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BALTIMORE

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BIOLOGICAL LABORATORY

EDITED BY

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AND

PROFESSOR WILLIAM K. BROOKS

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STUDIES

FROM THE

BIOLOGICAL LABORATORY

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A CONTRIBUTION TO THE EMBRYOLOGY AND PHYLOGENY OF THE PYCNOGONIDS.

By T. H. MORGAN, Fellow of Johns Hopkins University. With Plates I, II, III, IV, V, VI, VII and VIII.

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PART I.

In the year 1767 Karl Linné, in the twelfth edition of his Systema Naturae, described under the name of Phalangium a Pycnogonid, and here for the first time is the question raised whether the group is to be ranged under the Arachnids or the Crustacea. A hundred years elapsed and the problem remained

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unsolved; the group was placed now here, now there, now amongst the Crustacea and next amongst the Arachnids. Then Prof. Dohrn attempted the solution from the standpoint of Embryology, instituting a comparison "or even identity" between the Pycnogonid larva and the Nauplius, believing the Pycnogonids to have diverged from the Crustacea at this point. During the following twenty-five years opinions once more vacillated between Arachnidan and Crustacean affinities. Recently Prof. Dohrn and Dr. Hoek have each independently monographed the group, placing the morphology of the order on a very firm basis. Independently likewise they each reached the conclusion that the group is to be placed neither with the Arachnids nor with the Crustacea, but that these three groups have come down in parallel lines.

The early stages of the embryology of the Sea-Spiders have been practically untouched, and before any final decision as to the affinities of the group is to be made, these stages in the development should be known and take equal rank with Comparative Anatomy in disentangling the relationships of the group.

For many reasons the present paper attempts in no way to give a complete answer from the embryological side. The very great difficulties of a suitable technique had slowly to be overcome, and the time at command prevented a detailed description of the different organs arising from the germ-layers, so that much remains that might be done.

In the summer of 1889 material for work was collected at Wood's Holl, Mass. Through the courtesy of Prof. MacDonald I was enabled to collect and study at the station of the U. S. Fish Commission at this place. To Prof. MacDonald I am also indebted for many other kindnesses extended during my stay at Wood's Holl. Three genera of Pycnogonids, each with a single species, are to be found at this place, viz. Pallene empusa, Phoxichilidium maxillare, Smith (Anoplodactylus lentus, Wilson), and Tanystylum orbiculare. During July, August and September these are found with eggs. Pallene inhabits the hydroids (Tubularia, Pennaria) on the piles of the wharves, and is also common on the red sea-weeds below low-tide mark. The hydroids or sea-weeds as soon as collected were brought into the laboratory and worked over piece by piece. Each bunch was

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in turn swished rapidly backward and forward in a dish containing a small amount of water, so that the Pycnogonids were shaken loose and could be easily picked out. The other genera were more easily found, and on separating the masses of hydroids, etc., could be readily seen clinging to the stems. The males of Pallene carry on each pair of ovigerous legs a small bunch of eggs. Each bunch contains from one or two to fifteen or twenty eggs. The eggs of Phoxichilidium and Tanystylum are individually much smaller than the last, but are very numerous, so that the bunches are much larger, especially so in the former. Phoxichilidium carries several bunches strung along on the ovigerous legs of the male: the bunches are white and very conspicuous against the purple color of the adult. Tanystylum has smaller bunches of eggs, with the individual eggs larger than the former, and the masses are carried so that they form a circle of clusters held against the ventral side of the male.

The adults with eggs were put into alcoholic picro-sulphuric acid for several hours and then gradually carried through different grades of alcohol of increasing strength. Other methods of hardening gave far less satisfactory results, *i. e.* boiling water or Flemming's solution.

To prepare the eggs and embryos for study they were passed through absolute alcohol (one hour), turpentine (2–4 hours), soft paraffine (one hour), hard paraffine (1–2 hours). They were cut in paraffine, and fixed to the slide with albumen fixative; then back again through turpentine, absolute alcohol, 95 per cent, 80 per cent, 70 per cent alcohols to Kleinenberg's hæmatoxylin, where they remain for a very long time (12–48 hours); then washed fifteen minutes in acid alcohol and up again through the alcohols to turpentine and into balsam. In Pallene each egg was in many cases pricked with a very sharp needle before going into absolute alcohol. It is necessary to do this under a dissecting microscope. By these methods very excellent results were often obtained and, after many failures of other methods, was found to be the only satisfactory one. In Pallene the larger size of the egg makes a study of the earlier stages much easier, but the other genera have a much simpler development, and it seems better to give first an account of these.

To Prof. W. K. Brooks I am greatly indebted for help and suggestions during the work.

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Tanystylum and Phoxichilidium.

The eggs of Tanystylum measure .08 mm. in diameter (in preserved specimens), and those of Phoxichilidium .05 mm. In both animals there is a regular segmentation. In Plate III, Figure *a* is a surface view of an egg of Phoxichilidium divided into two equal parts; in Fig. *b* of an egg into four, but with the segments shifted around, and Fig. *c* of an egg into eight equal parts. Similarly Figs. *d* and *e* for the two and four-cell stages of Tanystylum. The eight-celled stage I did not obtain for this species. Fig. *f* is a surface view of an egg of Tanystylum at about the twenty-four-celled stage, and an optical section of an egg at this stage shows each cell to run from the periphery to the center of the egg, where they all come together at a point. Each cell is thus pyramidal in shape and contains a single nucleus.

In Plate I, Fig. 8, there is a section of an egg at a later stage than the last. This and the following sections are from eggs which were cut in paraffine. As the section is a later stage than the preceding surface view, the pyramids are narrower than before. Fig. 11 shows a somewhat older stage for Phoxichilidium. Very soon after this—perhaps after the next cleavage—a most important change takes place in the egg. I have not seen the actual change in the living eggs, but serial sections leave not the slightest doubt as to the process. Each nucleus divides radially into two, and this is followed by division of each pyramid into two parts in a plane at right angles to the radius of the circle lying in that pyramid. This is shown by Plate I, Fig. 9, for Tanystylum. Here it is seen that by a process of multipolar delamination the egg divides into two germ-layers—an outer peripheral circle of cells and an inner mass of cells. These latter soon round off and leave no trace of the pyramidal arrangement. In Fig. 9 one cell is still seen running from the periphery to the center. This section does not show a nucleus in the inner part. The inner cells I shall speak of as the entoblast and the outer circle as the ectoblast. This figure (9) is somewhat diagrammatic, inasmuch as only part of the inner cells is shown, for usually they are more closely packed together than here shown; and in this case it is due to a part of the section having broken away (redrawn in figure) and set free some of the inner cells. In Fig. 10 is a more accurate drawing

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of an egg of Tanystylum immediately after delamination has taken place.

Exactly similar changes were seen in the eggs of Phoxichilidium, but the figures just given serve in every respect for both species. The central as well as the peripheral cells continue to divide, but soon the entoblast cells lose their well-defined boundaries and the nuclei seem in part to disappear. The result is that we have in the center of each egg a granular yolk with scattered nuclei in it. Here and there is an indication of a cell boundary. During this time the ectoblast cells have divided tangentially and have become much smaller, yet at all times a distinct boundary remains between ectoblast and entoblast. Fig. 12 for Phoxichilidium shows an embryo at this stage.

It is here seen that the ectoblast cells over one hemisphere are somewhat higher than at the opposite, and I find this very constant in sections of both species at this stage of development. After this it becomes difficult to follow out the fate of the germ-layers. The outer cells become smaller and flatter to form the ectoblast and the inner cells arranged into organs, the most conspicuous of which is the digestive tract. There is a triangular invagination to form the stomodæum, and the proboscis appears between the first pair of appendages, which has now begun to form. These appendages are very conspicuous in surface views, where they project beyond the surface of the body. Between them appears the slightly projecting proboscis, and about the middle of the embryo are seen the second and third pairs of appendages, which are small and inconspicuous. Dohrn has given excellent figures of embryos at this stage, both in his earlier paper and in his later monograph. Soon after this the egg coverings swell up somewhat and the embryos finally break out of the egg case, so that the appendages now can straighten out.

In Plate IV, Fig. 9, is a surface view of a larva of Tanystylum as seen from the ventral surface. Dohrn speaks of this as the Pantopod-larva, and Hoek calls it a Protonymphon. Its general characters are shown in the figure. There are three pairs of appendages: the first pair chelate, with *the movable claw working outwards and downwards;* the second and third pairs have distally a sharp spine, in the middle of the limb a large segment,

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and proximally a prolongation of the body which is to be regarded as the basal segment of the appendage. Between the first pair of appendages the proboscis projects forwards, with the mouth opening at its distal end. Behind the base of the proboscis is seen the pair of ventral ganglia. From these the circumœsophageal commissure ascends upwards around the œsophagus, and laterally are given off on each side a pair of nerves to the second and third pairs of appendages. Dorsal to these ganglia—four in all, the two pairs being fused—is seen the outline of the mesenteron, which ends blindly behind. About the middle of the embryo and just back of the pair of ganglia is seen a pair of oblong masses, which are the beginnings of another pair of ventral ganglia. In the basal joint of the first pair of appendages is an opaque mass composed of large cells, from which runs out a duct towards the base of the spine, and Dohrn has traced it out to the very tip of the spine, where it opens to the exterior. This organ is a gland, and its duct is the long tube in the spine.

A dorsal view of the embryo shows the brain, just above and a little in front of the ventral ganglia. On its upper surface are a pair of small pigmented eyes. A transverse section through the body of this Pantopod-larva is shown in Plate I, 13. It passes through the center of the body and cuts below the large ventral ganglionic mass, passing through the base of the third pair of appendages. Above it cuts the posterior part of the brain. In the center of the section is the digestive tract. This is cut at a point where it is about to give off diverticula to the first and third pairs of appendages. The first of these is marked D^{I} and the second D^{II}.

Below the digestive tract is the second pair of ganglia, which sends out nerves to the corresponding appendages. Above the digestive tract the section at *B* cuts the posterior part of the brain. The cavity of the body has a few scattered mesoblast cells in it and a few bits of broken muscle fibers. These fibers in the living embryo served to connect the mid-gut to the body-walls. In the base of the legs are seen several cells, which at *M* are arranged as though around a central cavity. This cavity does not seem to connect with the mid-gut, and it seems very probable that it represents the body-cavity in the legs, and that the sur-

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rounding cells are of mesoblastic origin. In Pallene, as we shall see later, there are similar cavities, which are undoubtedly mesoblastic in origin. The mid-gut ends blindly behind, as is seen in other sections.

In Fig. 14 is shown part of a section through a more anterior part of the body. The section passes through the brain above and the first pair of ganglia below. These two are connected by the circumœsophageal ring. This commissure is composed of both cells and fibers. In the next section to the one figured a broad band of nerve fibers passes on each side from the brain to the ventral ganglia, and in Fig. 14 a few of these fibers may also be seen. In the middle of the commissure lies the cross-section of the œsophagus. Its lumen is triangular and its walls composed of a layer of cells, which are clear around the periphery of the triangle. Around the œsophagus are a few scattered mesoblast cells. From the brain, *B*, there is part of its substance which projects ventrally towards the œsophagus and is quite conspicuous in sections of the Pantopod-larva in this region. On each side of the brain are seen a pair of diverticula of the mid-gut. These go to the first pair of appendages and are traceable to D^1 of Fig. 13. The first pair of appendages are, as Dohrn has shown, innervated from the brain.

The section of the embryo following Fig. 13 gives a cross-section of the pair of simple eyes. A part of this section is shown by Fig. 15. In the middle line of this section above the posterior part of the brain, *B*, the ectoblast is thickened beneath the cuticle, and immediately on each side of the middle line appear the pair of eyes. Each is seen in section to be composed of three cells—two clear outer ones with large nuclei and an inner much pigmented cell. Around the eyes the ectoblast is quite thick, and on each side it sinks down slightly from the surface, suggesting that the eye may be here increased in size.

In Fig. 16 is a section through the third pair of ventral ganglia which we saw on the ventral side of the embryo posterior to the fused pairs of first and second ganglia. The section shows that at this place the epiblast is greatly thickened and the cells columnar, with clear outer portions. In the ganglion on the left of the section the cuticle seemed to dip down into the center of the ganglion, where it becomes extremely thin. This was not

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so clearly seen on the right-hand ganglion, but traces of it were seen here also. These structures I shall speak of as the Ventral Organs; and we shall come across them again in Pallene, where they are worked out in greater detail.

Pallene empusa.

There is a great variability in the size of the eggs on the ovigerous legs of the males. The average size of those which seem to be normal eggs is .25 mm., but often bunches contain one or more much smaller eggs, some of which seem to begin to develop. These very small eggs are most probably immature and accidental. Although the eggs of Pallene are much larger than in the preceding animals—having 125 times the volume—yet the yolk is almost transparent, so that nuclear divisions of the segmenting egg are easily seen from the surface. The adult is remarkably transparent and consequently most difficult to see as it rests quietly amongst the hydroids or sea-weeds. It seems that the eggs have also adapted themselves for purposes of protection in thus becoming transparent. More or less time necessarily elapses after the animals are collected before they can be examined, so that I have been unable to see the extrusion of the polar bodies.

The nucleus of the egg and its accompanying protoplasm is extremely large and situated near the center of the egg. Each division of the nucleus is accompanied by a division of its surrounding protoplasm, so that in surface views it is impossible to separate the one from the other. In the figures of Plate III the darker masses in each cell indicate the position of the nucleus and protoplasm, but for the sake of brevity I shall speak of this simply as the nucleus. In the unsegmented egg the nucleus is seen to elongate and to divide into two halves, and this is immediately followed by division of the egg itself into two parts. The first furrow divides the egg into two unequal segments. Fig. *A*, Plate III, shows an egg after the first division. Each segment contains a single nucleus. (Hoek has made a different observation from this—see *infra*.) After this first segmentation the segments flatten somewhat and remain so during the resting period. The second division came in about three-quarters of an hour (to an hour) after the first. The

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nucleus of the smaller segment—the micromere—divided first and then the segment itself into equal parts. This was followed five minutes later by the division of the nuclei of the large segment—the macromere—and then the macromere itself.

In Fig. *B* an egg is shown as seen from above after this second division, which is indicated by 2—2. The planes of segmentation of the micromere and macromere are drawn in this figure as though they coincided, but this is not the rule. The plane of segmentation of the micromere may be turned at as much as 30° (or exceptionally almost 45°) to that of the macromeres. I think it should nevertheless be considered as one and the same plane and one and the same division, in spite of individual variations.

The segments again flatten together, and after an interval of about an hour the third division commences. The plane of division is at right angles to the last and is shown by Fig. *C*, in which the egg is seen from above. Again the planes of micromere and macromere do not quite coincide; the numbers 2—2 indicate the second division and 3—3 give the plane of the present (third) division. This plane is a zigzag line lying between 3—3. If we examine the opposite pole, the lower, of an egg at this stage, we find the large macromeres come together, as shown in *D*, although there are many differences of detail in this respect. So far the planes of division of micromere and macromere have been supposed to coincide or to be referred to the same division plane, but after this stage is reached (8 segments) the planes cannot be considered identical. About an hour after the last division the fourth rhythm comes on. This division plane is shown for the macromeres in side view in figure *E*, by the line 4—4, and is seen to be parallel to the first plane of segmentation, 1—1. The four macromeres become eight. About the same time—maybe five minutes before or after—the micromeres divide. In Fig. *E* they are drawn after division has taken place, and Fig. *F* shows how these cells are divided, the curved lines indicating twin cells. As we see from the very nature of the division of the macromeres, it is impossible to have their plane of division to correspond with that of the micromeres, and the division lines of the latter lie on a plane nearly at right angles to the plane of division of the micromeres. We have now eight macromeres and eight micromeres. After a resting period of about an hour the fifth rhythm begins.

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The nuclei of the macromeres of Fig. *E* show by their elongation how they are to divide, and the plane of division lies between them or at right angles to the last division plane. Fig. *G* shows the egg after division into eight micromeres, but the micromeres had not as yet divided and remain eight in number. Soon, however, they do divide, but no definite plane of division for all of the segments was discovered. There are now sixteen macromeres and sixteen micromeres.

The next plane of segmentation of the macromeres—at the sixth rhythm—comes in after another resting period of an hour. This is shown by Fig. *H* at 6—6, 6—6. The two planes are at right angles to the last two (5—5) and are parallel to the first and fourth (1—1, 4—4). I did not see the sixteen micromeres divide into thirty-two, nor could I trace them further. There are thirty-two macromeres and sixteen micromeres, or in all forty-eight cells. After an hour's interval the macromeres lying between the planes 1—1 and 4—4 in Fig. *H* were each seen to have two nuclei, but this did not seem to be the case with the macromeres of the lower pole. The egg had been under observation for many hours and did not develop further, so that this last attempt at division in the upper macromeres may have no special significance.

Besides the general facts of segmentation as just given, several interesting variations in the method of segmentation of the egg were clearly made out. In the example given above the micromeres divided first (at the second segmentation), giving two micromeres and one macromere. In two other observations this was also true, but in three other cases the macromere divided five minutes before the micromere. And again in the last case the two macromeres divided into four before the two micromeres divided into four, so that the greater amount of yolk of the macromere did not seem altogether to retard segmentation. In several later stages (thirty-two macromeres and sixteen micromeres) the rhythm of macromeres and micromeres given above did not so closely correspond, but the micromeres seemed to drop behind.

If the accumulation of yolk has been a very recent event in the egg of Pallene—and the variations in size may bear this interpretation—these differences in the method of segmentation may

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be due in part to the segments of the egg struggling to give synchronous beats at each rhythm, but being hampered by the presence of the yolk. If the first plane of segmentation of Pallene corresponds with the first plane of division of Phoxichilidium and Tanystylum, and *à priori* this seems most probable, is it not conceivable that the acquisition of yolk to one-half of the egg might cause great changes in the synchronism of segmentation of the two parts? and if so, it is easy to imagine that we have here an egg in a period of variation, and that one or another of the above changes may become fixed for the species.

Later stages of segmentation than those described are difficult to follow or to distinguish between macromere and micromere of the upper pole, but in general the cells around the upper pole are smaller and more numerous than at the lower. . Fig. *J*, Plate III, shows the ventral (or macromere) hemisphere of an egg at a later stage than any of the preceding. The outer ends of the cells, those seen in the figure, are polygonal, and the nuclei lie very near to the surface. The whole cell is pyramidal in shape, with its apex at the center of the egg and its polygonal base at the periphery of the egg, with the nuclei in the base of the pyramids. Each nucleus is still accompanied by a surrounding mass of protoplasm, and it is without doubt this protoplasm which is seen from surface views. At this time the cells of the lower pole rarely divide, and remain for a long time almost constant in size and number, but the cells at the upper pole undergo rapid changes; and our attention must now be turned almost entirely to that region.

The next change which we can see in surface views is at the upper pole, where a whitish opaque area is forming, and which may be profitably compared to the similar formation in spiders' eggs and called there the Primitive Cumulus. This is due to an accumulation of cells at this point, where an invagination is about to take place to form the stomodæum. Soon there are other opaque areas formed at the surface, which are seen to occupy definite positions and are the thickenings for the brain, ventral ganglia and appendages. These are shown in Figs. I and II, Plate IV. In Fig. I we have a surface view of the head region of a young embryo. In the upper part of the figure are first the two oval thickenings to form the brain or supra-œsophageal ganglion.

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At this stage they just touch on the middle line. Below this and on the middle line is the thickening of the stomodæal invagination. In the center is a triangular-shaped cavity—the cavity of the invagination. The base of the triangle is towards the brain. On each side are the thickenings of the first pair of appendages. In the lower part of these is a slight depression, which indicates the line between the claw and the next segment against which it works. Posterior to these various thickenings appear two rows of opaque areas, the nerve ganglia. Three pairs of these are seen in this figure, the first two being somewhat smaller than the third. On each side of the last pair appear part of the thickenings of the first pair of ambulatory legs (the fourth pair of appendages).

Fig. II is a continuation of the last figure, and shows the ventral and posterior part of the same embryo. The upper pair of ganglia are the second pair, and were seen in Fig. I, and the second pair of this is the third of the last figure. On each side of this third pair of ganglia are again the first pair of walking legs. Two more pairs of ganglia follow this third. On each side of the fourth and fifth pairs of ganglia appear the second and third pairs of walking legs. An embryo at a stage later than the last is shown by Fig. III. Here the embryo has elongated in an antero-posterior direction, so that the figure shows only the ventral side of the animal. Fig. IV is a dorsal view of an embryo at the same stage. These figures show that the region about the stomodæum has grown forward and has the opening of the stomodæum at its distal end. This outgrowth is seen in the figures as a forward prolongation of the embryo on the middle line. On each side of it are seen the first pair of appendages, which have also grown forward.

On the ventral side of the embryo appear on each side of the middle line five pairs of large ganglia (see Fig. III). On each side of the last pairs appear the three pairs of walking legs, which are longer than in the last figure, are bent, and stand out from the surface of the embryo. Posterior to the last pair of ganglia the embryo has a thickened mass of undifferentiated substance. In a dorsal view (Fig. IV) we see the brain, and behind it the large yolk mass of the embryo. The two halves of the brain have come together, and each half is slightly bi-lobed. Around

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the periphery of the yolk appear the six pairs of appendages, and behind, the thickened posterior mass of the embryo.

Returning to the ventral view of the embryo (Fig. III), there is seen a most interesting structure in the center of each ganglia. Each is an invagination of the surface into a ganglion, and these invaginations are elliptical in outline, with the long axis corresponding to that of the embryo. These structures I shall call the Ventral Organs, and, by means of serial sections, we shall later study them in more detail. In the next stage, shown by Fig. V, the embryo is more oval in outline. The appendages have also elongated and become more bent. The posterior pair have begun to grow forward between the more anterior pairs. The most important change is in the fusion of the first two pairs of ganglia, which now appear as one pair of rather large ganglia. This pair of ganglia still shows its double structure by the presence of two pairs of ventral organs. The third pair of ganglia also shows a pair of ventral organs, but the more posterior ganglia are covered by the posterior appendages, so that these ganglia cannot be seen from the surface.

After this stage is passed the appendages grow enormously in length, and the embryo becomes flattened from side to side. A figure of an embryo at this stage is shown in side view in Fig. VI. The first appendage is chelate, and has a joint near its base, and one also sees it has moved more dorsally to the proboscis. Beyond and beneath this appendage appears the proboscis, which has much elongated. Near the base of the appendage is seen part of the brain. This figure also shows that the yolk mass is continuous along the center of each ambulatory limb. The three pairs of legs are much bent, and each ends in a spine-like process. The body is seen to end behind in a knob-like projection. The ventral ganglia are shown between the bases of the legs, and the height of each at this stage is about equal to its length. In older embryos the yolk begins to disappear and the sides of the embryo to become thicker. After this the embryo lengthens a great deal, the appendages grow much longer and become segmented. Another pair of appendages appears behind the third pair of walking legs, and the knob-like projection at the end of the embryo is pushed more dorsally, to form the rudimentary abdomen. Four pairs of eye spots appear over the posterior end of the brain.

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After these changes have taken place we reach a stage shown by Figs. VII and VIII. The first figure is a ventral view of the embryo at a time when it is ready to leave the parent. The three pairs of segmented walking legs have become straightened out at the sides of the body. In the figures only the proximal ends are shown. The fourth pair of walking legs appears at the posterior end of the body. The first pair of appendages—the cheliceræ—are now attached quite dorsally to the proboscis, which appears between and below them. Each has three segments, including the terminal one, which acts together with the second to form the pincers. On the sides of the body, just in front of the first pair of ambulatory legs, are a pair of projections, one on each side. These are the beginnings of the third pair of limbs—the ovigerous legs. I have seen no traces of the second pair of appendages in the ontogeny of Pallene. Five pairs of large ganglia lie along the body, and in addition a small sixth pair. The first pair, as we have seen, is double, and composed of the first and second pairs. The next pair, the third, is between the first pair of ambulatory legs; the fourth pair of ganglia lies between the second pair of legs; the fifth near the base of the third pair of legs, and the sixth, each of whose ganglia are smaller than those in front, at the base of the fourth pair of legs. The small, seventh pair of ganglia belongs to the rudimentary abdomen, and does not lie in a plane with the more anterior ones, but, like the sixth, is dorsal to the one in front of it, as partially shown in the figures. The œsophagus is seen to start at the distal end of the proboscis and to disappear in the body, where it is not easily followed on account of its transparency. The prolongation of the mid-gut into the legs is not shown in the figures, except for the last pair of immature legs.

In Fig. VIII is a dorsal view of the same embryo. Four pairs of large ventral ganglia are seen, and in addition the small rudimentary ganglia of the abdomen. The fused first and second pairs are hidden by the brain. On the surface of the brain are seen four small pigmented eyes. The œsophagus is triangular in shape in cross-section, with the broad base turned upwards, and this base is seen from the surface. At the posterior end of the animal the rudimentary abdomen is seen, and at its end is the opening of the anus.

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Internal Changes.

Sections of the early stages of segmentation give little information in addition to what we see from surface views. They show that each nucleus is contained in a separate segment, and that many of these segments run to the center of the egg. Those cells derived from the first micromere cannot reach to the center of the sphere.

Fig. 1, Plate I, is a section of an egg at the stage when there are thirty-two micromeres. The upper part of the figure is probably in the region of the micromeres. In the center of the egg appears a clear cavity without yolk, but this is not constant for all eggs. In some it is certainly absent, but I am unable to say whether or not it is caused by hardening reagents. What seems to be a similar cavity is found in the eggs of some spiders. The whole egg is divided up into the segments in the form of pyramids. Only some of these in this figure can be traced to the center, but other sections show each pyramid of the macromeres to have its apex at or near the center of the egg. The nuclei are situated around the periphery of the section, and the protoplasm, which invariably accompanies each nucleus, sends out fine pseudopodial filaments into the surrounding yolk.

Fig. 2 is in the same stage as the last, and undoubtedly passes through the micromeres at the upper pole. These micromeres and their nuclei are seen to be smaller than the cells elsewhere, and the pyramids are seen to fall short of the center of the egg. There is no central cavity present in this egg.

Sections of eggs somewhat older than the last show that the nuclei of the upper pole have rapidly increased, and that they have migrated to the surface of the yolk, which loses its pyramidal structure over the upper surface. The protoplasm surrounding each nucleus comes into contact with that of surrounding nuclei, though how closely such fusion is formed I cannot say. Such a condition is shown in Fig. 3, where the protoplasm forms a cap over the upper surface of the yolk. The nuclei in this section are abnormally large, which is probably due to the hardening reagents, but the section seems normal in other respects. The lower area of the same egg shows three or four scattered nuclei near the surface of the yolk. At this stage of development there is a single layer of cells at the surface of the yolk. Between this

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stage and the next which I have figured there is a gap. In this next section, Fig. 4, we see the number of nuclei at the upper pole to be more numerous than before and much smaller. The protoplasmic cap has become larger and its protoplasm is for the most part without the amœboid processes of the last figure.

A very important change has taken place between these two stages, viz. the formation of an inner layer of cells. Within the area of the cap appear a few somewhat flattened cells in Fig. 4, and these send out processes into the underlying yolk. Two of these cells are shown in this figure. I have not made out any definite arrangement for these cells, but they seem to lie only under the upper surface of the embryo. Where these cells come from or at what time they appear must in part be a matter of conjecture, but much light is thrown on their possible origin by a study of somewhat older stages. In Fig. 5 is a section of such a stage. The number of nuclei in the outer germ-layer has nearly doubled, and the area itself covers a much greater surface of the egg than in the last stage. Under the blastoderm are seen five inner cells with their pseudopodial extension. The larger number of these cells here than in the last figure is due in part to the greater thickness of the sections. At the lower pole are two nuclei. These belong to the outer germ-layer, although they show (as did all the outer at an earlier stage) the protoplasmic extensions into the yolk. This section itself throws little light on the cells of the inner layer, but in other sections at a similar stage I have found what seems to furnish the solution of the problem. From such sections I have drawn Figs. 6 and 7. These are taken from the periphery of the cap of cells at some such point as *P* in Fig. 5. Fig. 6 shows two cells which have just separated from the outer layer of cells, and we also see beyond them a single mass of protoplasm with two nuclei which presumably have just divided. I have not seen any karyokinetic figures in nuclei dividing in this direction, and it is possible we have here a direct division, as Heider has recently shown in Hydrophilus at a similar stage of development, although it is equally as possible it is due to poor preserving agents. Again, in Fig. 7, we see a single mass of protoplasm with two nuclei, the result of division. These I believe to give a clue to the origin of the inner cells of the preceding figures, and to point out that they too have had a similar origin from the outer layer.

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Keeping before us the process of delamination in Phoxichilidium, etc., I think we may regard these inner nuclei of Pallene to have come from the outer cells by delamination, and even that we may push the comparison a step farther and consider that each cell of the outer layer to have given rise at one time in its history to an inner cell, and then that the outer cells continued to divide tangentially to form the blastoderm. The reasons for such a belief are these:—In cross-section the number of inner nuclei are slightly in excess of the peripheral nuclei of the lower pole (see Figs. 4 and 5). As the outer cells of the upper pole were at the beginning more numerous than the peripheral cells of the lower pole, we ought to get, if the hypothesis be true, exactly what we do find. Further, at the periphery of the blastoderm, where the inner cells of the lower pole are added on, we can always see such a method of multiplication taking place. The differences between this process and that in Phoxichilidium are these: that multipolar delamination does not take place simultaneously in all the cells at once, but in Pallene slowly progresses as the cap of cells makes its way to the lower pole of the egg, incorporating into itself as it goes the outer cells, which cells, as they are added on first, give off an inner cell.

After this stage we pass to older embryos, where these two layers—ectoblast and entoblast—begin to differentiate into organs. The first to appear is the stomodæum, which results from an invagination of ectoblast. This is shown by Fig. 17, Plate II. Here it is seen the ectoblast at one point has pushed inwards, and around the periphery of the invagination appear several cells with branching and anastomosing pseudopodia. These I believe to be the first appearance of the mesoblast. A few of the cells drawn in the figure belong without doubt to the entoblast, and at this stage it is difficult to separate the two. The section passes longitudinally through the embryo, and just ventral to the stomodæum there is a thickening of the ectoblast to form the first ventral ganglion. Under the ectoblast are found much branched entoblast cells, which are still comparatively few in number. Whether the circumstomodæal mesoblast comes from ectoblast or entoblast I am unable to say. Dorsal to the epiblast the epidermis continues conspicuously for a short distance and then becomes exceedingly thin.

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From this stage we pass to embryos of the age represented by Figs. I and II, Plate IV. The first figure, 18, is a cross-section through the stomodæum of Fig. I (see line 18 of the figure). The invagination is deeper than in the last section, and its lumen is closed. The ectoblast nuclei of its walls are two layers thick. On each side of this central invagination is what appears to be a lateral invagination. These supposed invaginations have given me endless trouble, and even now I feel some uncertainty as to my interpretation of them. On each side of these is seen the thickened ectoblast of the first pair of appendages. The lateral invaginations are, I believe, caused by the growth of these appendages, which tend to grow outwards as they increase in size; but are prevented from doing so by the egg coverings, and the result is that the ectoblast becomes folded on itself to make room for the growing appendages. This view is strengthened by the presence of somewhat similar invaginations at the side of the other appendages. This part which is pushed in would correspond to the dark furrow between each appendage of Fig. I and the cells forming the stomodæum. The mesoblast around the stomodæum and under the appendages has increased, and is now clearly distinguishable from the inner covering of the entoblast, which lies only at the periphery of the yolk, and between it and the mesoblast.

In Fig. 19 we have a section from the same series as the last, but more anterior and through the region of the brain corresponding to line 19 of Fig. I. The section is entirely in front of the stomodæum, and cuts the two brain thickenings of the surface view. Here again the ectoblast is seen to be distinctly folded, and this folding (invagination!) is directly continuous with the last. At first sight this seems nothing more than a forward continuation of the last groove, but it is not clear why the folding of the appendage should exert any influence over that part of the brain. Again, it might be interpreted as folding due to the brain, but I can see no good reason why such thickenings (not outgrowths) should produce the groove. There is one other possibility, viz. that these may represent invaginations into the brain itself. Against such a view is the absence in surface views of any such invaginations, and also that the folds are directly continuous with and quite similar to the groove between the stomodæum and the

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appendages, so that I cannot hold this either as a true solution. Beneath these ingrowths appears the pair of thickenings for the brain, which are seen to be continuous across the middle line. On the inner surface of these lobes are seen a few entoblast cells, as shown.

The next figure, 20, passes through the middle of the brain, but is farther forward than the last (see line 20, Fig. I, Plate III, which indicates the plane of the section). The cells of the ectoblast seem to pass into those of the brain. Sections still farther forward, where the two lobes are still cut, show the brain thickenings on each side and separated from each other. We see in surface views of embryos at this stage that the ventral ganglia have appeared, and Fig. 21 is a section through two of these—the first pair. Not only do we see that the ectoblast has greatly thickened in two places on each side the middle line, but the outermost cells of each mass show certain peculiarities. They are much elongated at right angles to the surface, and their outer points come together at the middle point of the surface of the ganglion. The left-hand ganglion in the figure is cut through this central point, and the ganglion on the right a little to one side. The nuclei lie in the inner parts of the cells, and the outer parts of the cells are clear and transparent, while their inner ends are more granular. This difference in the parts of the cells is very constant through the later stages. The cells form a structure which I have called the Ventral Organs.

In Fig. 22 is a section of a pair of ventral ganglia from an embryo at stage III. Here there is in the middle of each ventral ganglion a wide invagination lined by columnal cells with their clear outer portions turned towards the cavity of the invagination. The nuclei in the cells are larger than those in other parts of the ganglia, and are seen quite often in process of karyokinetic division. The spindles of the dividing nuclei are in some at right angles to the long axis of the cells, and in others parallel to the axis. A narrow connection of small ectodermal cells runs across the middle line from ganglion to ganglion.

The next figure, 23, is from an embryo at about stage V. The outer edges of the invagination have turned in until they have nearly met above the cavity of the invagination. In other respects the section is similar to the last.

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At the next stage, shown by Fig. 24, the arching over is completed and fusion has taken place, so that there is a cavity in each ganglion. The cavity does not run through the whole length of each ganglion, but lies only in its middle portion. There is no communication between the cavities of different ganglia. In this figure each ganglion has increased in size, and the cells have become more numerous, and, further, the neighboring ganglia are connected by a cross commissure of fibers. Further, the nuclei of the outer cells, those lining the cavity, are now more like the nuclei of the ganglion cells. Later stages show that the central cavity of each ganglion disappears, although they seem to persist for quite a long time after they have been enclosed by the ganglia. That this structure may have represented an organ in some ancestral form I believe possible, first, because it is distinctly marked, differentiated, before any invagination takes place; secondly, because their cells are histologically distinct from the surrounding ectoblast and from the cells of the ganglion beneath, and lastly, from the arrangement of the cells, which suggests a sense organ comparable to the simple ectodermal organs of many animals. It also seems probable that we have exactly similar structures in the Pantopod-larva of the other Pycnogonids.

Returning to stage III we have a cross-section of the body drawn in Fig. 25. The section passes through the middle of the body in the plane of a pair of walking legs. The large mass of yolk is still seen filling the upper part of the embryo. Over the ventral surface of the yolk the entoblast cells form a continuous covering and are indicated by *N* in the figure. At the base of the appendage the entoblast sends out a prolongation which is filled with yolk, while the entoblast cells are here higher and more closely packed than elsewhere. Into each of the six walking legs is a similar protrusion of entoblast from the mid-gut. Beyond these diverticula *there is in each leg a definitely marked cavity in the mesoblast.* In Fig. 25 the surrounding mesoblast is shown by *M*. As the prolongations of the mid-gut push into the legs these cavities cannot any longer be made out, and either become lost or are too complicated to follow out. Besides these cavities, which would seem to be body-cavities, we shall come to others later in development which seem to be

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schizocoels. The surrounding mesoderm of these latter is irregular and quite different from that around the body-cavity proper. The ectoderm over the appendages is thicker than elsewhere. The ventral ganglia at this stage have in most cases the ventral organs, although in this figure none are open and they have closed rather early. The ectoblast of the dorsal surface is quite thin and the mesoblast has not appeared in that part of the animal.

In Fig. 26 is a drawing of a cross-section through the head of an embryo at a stage a little later than III, but not so late as V. The section passes through the brain above and the second pair of ganglia below. Between the brain and the ventral ganglia the stomodæum is seen. Its lumen is seen to be triangular in outline, with its base turned upwards. On each side of the stomodæum appear cross-sections of the first pair of gut-diverticula. A single row of entoblast cells surrounds the central mass of yolk. Scattered mesoblast cells are found around and between these different structures, leaving here and there openings between the cells. Such schizocoels are shown by *S*, *S* in the figure.

Going on to stage V we find that sections give little more than was seen in the preceding figures. The prolongations of the mid-gut into the legs have grown longer, and, as seen from surface views, the first and second pairs of ganglia have fused.

In stage VI the yolk mass continues to fill the central and upper part of the embryo, but it now begins to decrease in quantity. This may be due in part to the fact that part of it becomes digested and built up into the tissue of the embryo, and in part to its extension into the legs.

A cross-section of an embryo at a stage a little older than VI, in a plane of a pair of limbs, is shown in Fig. 27. The ventral ganglia have increased enormously in size and show a large amount of punkt-substance. In the middle of the section lies the mid-gut with its contained yolk, which has decreased very much as compared with preceding stages.

In Fig. 28 is drawn a section of another embryo, at about the same stage as the last, but cuts the embryo between a pair of legs. Above the large ganglion is seen the digestive tract, which is completely separated from the body-wall by a series of

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schizocoels. The uppermost of these, lying in the middle line, is the heart, *H*. At the sides of the mid-gut appear two well-marked cavities, and a third between the nerve ganglion and the outer wall. Also below these are two or more spaces, but these latter, and perhaps some of the others, are artefacts. The mesenteron is covered on its outer side by a distinct layer of mesoblast cells. Cross-section of some of the legs lying below the body is also shown in the figure.

Between stage VI and stage VII–VIII there is a considerable gap. During this period the embryo has lengthened and the fourth pair of walking legs has appeared. The rudimentary abdomen has been pushed up dorsally and the proctodæum invaginated until at stage VII it has opened into the mesenteron. The eyes are now seen, but at stage VI they are seen in sections as four thickenings of the epiblasts above the brain. In stage VII–VIII we find by sections that the yolk has almost completely disappeared from the digestive tract. Cross-sections also show the large nerve ganglia, the mid-gut with its diverticula, and on the dorsal part of the mid-gut the tubular heart running from the first walking leg to the third.

Fig. 29 is from a cross-section through the posterior part of the embryo and cuts the mid-gut at its juncture with the proctodæum. Below the digestive tract *D* is a pair of small ganglia, V^7, which are the ganglia of the rudimentary abdomen. On account of the shifting of the abdomen to the dorsal side these ganglia have been carried above the last ventral ganglia, which are shown at V^6. But this is not all, for this sixth pair of ganglia has likewise been affected by the shifting of the abdomen and in turn lies above and back of the fifth pair of ganglia. In the sixth pair of ganglia, as shown in the figure, we have evident traces of the ventral organs. In the upper part of the section, and on each side of the middle line, are the diverticula of the fourth pair of walking legs. A few scattered mesoderm cells appear in the cavity of the body. The embryo must leave the male at about this time, for I have never found older embryos attached and only exceptionally ones so old as these last figured.

Comparisons.

Comparing the embryology of the two types represented by Phoxichilidium and Pallene, we find that most of the changes of

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the latter may be explained by considering it an abbreviation of the type represented by Phoxichilidium. An exact comparison of the segmentation of the two forms would be of interest; but, in order that such a comparison should be of value, the exact orientation of the segmentation plane would be essential, and such observations are wanting.

It would seem *à priori* most probable that the first planes in each must correspond, and that the unequal segmentation of the egg of Pallene has been caused by the greater accumulation of yolk in that part of the egg which corresponds to the macromeres. It is also probable that the smaller segments—the micromeres—correspond either to the anterior or to the ventral part of the embryo(?). Which of these is correct it is difficult to say. The embryo differentiates earlier in what corresponds to the anterior region of the adult than over the whole ventral surface, which suggests that the smaller cells may have adapted themselves to this early differentiation; but it seems equally possible that this differentiation may be due to phylogenetic laws in this particular case rather than to any mechanical connection with the micromere differentiation. So that for the present the question must remain unsettled until by actual experiment (which would not be difficult) the orientation of the segmentation planes be determined.

There is an observation on the segmentation of the egg of Pallene brevirostris by Hoek, which was mentioned in the previous description of segmentation of Pallene empusa. He says: "Le fractionnement commence par le fractionnement du noyau, et seulement après que quartre noyau sont formés, un premier fractionnement devise l'oeuf en une partie plus grand et une autre beaucoup plus petite. Chaque partie contient deux noyaux qui dans le plus petit segment sont plus rapproches l'une de l'autre que dans l'autre segment."

I cannot believe this a correct observation in the light of what I have observed over and over again in Pallene empusa. There is a stage which corresponds exactly with the description, but the egg itself has previously divided into two with a single nucleus in each segment, and subsequently each of these nuclei divides into two just before the segment itself divides, and at the same time the first furrow becomes more distinct again as the

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segments round off to form the second furrow, and this seems to be the stage which Hoek has described as the first segmentation. Nor does it seem probable that the differences of our observations are due to their having been made on different species, for in each case the egg is approximately the same size.

The changes which take place in the formation of the endoderm of Pallene may, I believe, be referred to the simpler changes of Phoxichilidium, etc., and furnish in this respect an excellent basis for further comparison with other forms having much yolk present. In Phoxichilidium the pyramidal segments divide into an outer and an inner cell, while in Pallene the nuclei alone divide; and although delamination is still multipolar, it is not synchronous over the egg. A further comparison of the mesenteron I am unable to give, owing to lack of complete observations of the changes of Phoxichilidium. In both types the œsophagus invaginates with a triangular lumen, and in each the proctodæum forms later in development.

The few observations I have made on the ventral organs of Tanystylum leave no doubt that it is the same structure as in Pallene. Prof. Sedgwick has described in Peripatus paired ventral organs which correspond in number and position with the pairs of ventral ganglia. Comparing Fig. 21, Plate II, with his figure for the ventral organs of the jaws of Peripatus (Studies Morph. Lab., Camb., Vol. IV, Pt. I, Plate 10, Fig. 4), a striking similarity is seen, and, in addition to this, he says in his account of the ventral organs that they are slightly invaginated from the surface. Whether these structures are in any way related it is impossible to say, but it is worth while to call attention to the close similarity both in position and structure between these organs in the two groups.

A comparison of the appendages and their order of development is of interest. Prof. Dohrn has most carefully and fully worked out the transformations of the larval forms, and through his skill we have a very thorough knowledge of the transformation of the embryo. According to his account the six-legged larvæ of the Pycnogonids with the Pantopod-development pass into the adult condition by the body elongating behind the last pair of appendages and the walking legs appearing from before backwards, in much the same way as Prof. Claus believes the

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typical Nauplius to pass into the adult. During the growth of the walking legs the second pair of appendages of the Pantopod-larva has increased a little in size, but the third pair loses its spines and the whole appendage becomes a simple prolongation of the embryo. At a later stage, when the larva has increased in size, this third pair grows out again to form the ovigerous legs. In Pallene we have seen that the first, fifth and sixth pairs of appendages appear simultaneously in the embryo. The seventh comes in very much later, and the third after the seventh. The second did not appear at all in the ontogeny, so that in the young Pallene the only appendages in the young embryo that correspond to those of the Pantopod-larva are those of the first pair. The development of Pallene has become so much abbreviated that there is only a trace of the true Pantopod-larva found in its ontogeny.

Phylogeny.

There is a general agreement that the Pycnogonids are to be placed within the large group of Arthropods, but after this there is the greatest divergence of opinion as to which group of Arthropods they are most nearly allied. In general there are two prominent categories to which all or nearly all of these theories may be referred. One lot of workers believed in a Crustacean relationship, and another lot placed the Pycnogonids amongst the Arachnids. It is needless to give here the reasons assigned for these opinions, as Prof. Dohrn has given in his monograph of the Pantopoda of the Gulf of Naples a most complete and exhaustive bibliography of the literature. The two most important views which we have at present are those of Dohrn and of Hoek, but, as the latter agrees with much that the former has given, we may consider the two together.

Prof. Dohrn's earlier view of the relationship of the Pantopod-larva to the Nauplius he has completely given up in his more recent papers. In an appendix to a paper in the *Archives de Zoologie experimentale*, Dr. Hoek gives a summary of Dohrn's recent theory as to the phylogenetic position of the Pycnogonids, and has at the same time contrasted his own views with Dohrn's, so that a translation of a part of this review will best bring out the resemblances and differences of their opinions.

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"For the Crustacea Dohrn rejects the Nauplius theory (of Fritz Müller and Claus) and adopts that of Hatcheck, who believes the Crustacea to have descended from parents which had the form of Phyllopods, just as these Phyllopods have in turn descended from Annelids. From these same Annelids, according to Dohrn, the Pycnogonids have come down. The number of their segments was originally more numerous than we now see them, the presence of a pair of rudimentary ganglia in connection with the last pair of ventral ganglia allows us to add an eighth pair of appendages, and, together with the first, all of these appendages were originally homotypes. . . . They received diverticula from the intestine . . . and each appendage enclosed within itself a reproductive organ with a special genital opening. (The so-called excretory organs of the palps and of the ovigerous legs are the rudimentary sexual organs of these appendages.) The appendages were much shorter than we now see them, the heart showed many openings, etc. The supposed ancestor that Dohrn reconstructs might be very well compared to an Annelid.

"We also notice that Dohrn persists in his opinion, published in his work of 1879, that the Pycnogonids have a parentage neither with the Arachnids nor with the Crustacea (they have developed by the side of the last and altogether independently). In this I am in accordance with Dohrn. I was struck in the first place by the very general presence in the Pycnogonids of a characteristic larval form (the Protonymphon), and its presence suggested to me the idea of their descent from an ancestor resembling somewhat a larva which took its place by the side of the hypothetical ancestor of the Crustacea—the Nauplius—and this by the side of a third (the ancestor of the Annelids), and that all three groups would have descended from a common ancestor. I had tried to obtain in this way an explanation of the affinities of the three groups of animals (Annelids, Pycnogonids, Crustacea). Dohrn, on the contrary, took into account the larval forms, but constructed a phylogeny comparing together those animals having a considerable number of segments. It is true I do not wish to deny that such a method of looking at it has not just as good a basis of reason, only it appears to me at present that it can be defended just as little, and certainly no better than the larval theory.

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"No one affirms that the intestinal diverticula which now in Phoxichilidium and Nymphon penetrate into the proboscis were originally [ancestrally] in the palps and ovigerous legs. The homology of the glandular organs of appendages II and III with parts of the sexual organs is not based on any observation. . . . But since I believe there are certain things which lessen Dohrn's arguments in favor of the Annelid theory, I believe that at bottom and the contents of pages 82–115 of his work confirms this opinion, the ideas of this author and of mine upon the phylogeny as well for the Crustacea as for the Pycnogonids are not very different. At first I saw only the weak side of Dohrn's theory, that he does not give any explanation of the almost universal presence in the ontogeny of these animals of a characteristic larval form (the larval Protonymphon, which for myself is a true primary larva in the sense of Balfour). So that I cannot adopt a theory which causes to arise a form of Arthropods with many segments from an Annelid with the same number of appendages; but such is not the opinion of Dohrn, as clearly expressed in the following paragraph: 'Thus the larva of the greater part of the Pycnogonids may be regarded, with a grain of salt, as a form much like the ancestor; but if, on the other hand, the absence of an anal opening, the pincers of the first pair of appendages, the long claws with their accessory spines, the structure of the proboscis with its triturating apparatus and its ganglia, and the form of the cutaneous glands with their integumentary hairs, can only be considered as having been acquired in a much later stage, and transmitted to larval life where they are found at present; but what remains in the larva that we may regard in reality as inherited from its original condition? Nothing but the nervous system, *i. e.* the brain and ganglia, an intestine, three pairs of appendages of a form modified according to circumstances, and two eyes! But these are attributes which one finds equally in the larva of Annelids with three segments! And if then we take into consideration that at one time the body of the Pycnogonids, as we believe, showed a great uniformity in the segments (and the generative organs prove this), that on the one hand there was a concentration and a differentiation, and on the other hand a reduction in number of segments, these two conclusions lead us to admit a direct descent of the Pycnogonids from ancestral

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Annelid forms which were homonomously segmented. Then the larva of the Pantopods came from a larval form with Pantapod characters added on, but at the same time a larva which never had an independent and mature existence.'"

Whatever Hoek's position may be with respect to the Pantopod-larva, he agrees with Dohrn in the most important part of the latter's theory that the Pycnogonids have come down from the Annelids independently of the other groups of Arthropods. In examining the preceding account we may take it up for the sake of clearness in two parts, the first pertaining to the ancestry of the adults, leaving the larval form out of account, and the second part where the meaning of the larval form will be considered.

I believe that if the account I have given of the early stages of development be even approximately correct, there is little or no ground for a comparison between Crustacea and Pycnogonids—certainly not with any existing forms. The multipolar delamination of the endoderm in the Pycnogonids has no homologue amongst the Crustacea, nor is there any special similarity in the formation of the organs. There seems to be no trace of gastrulation like the Crustacean in the ontogeny of the group. And if we have reason for rejecting a relationship between the Pantopod-larva and the Nauplius—and I believe with Dohrn that we have—there remains nothing in common to the ontogeny of the two groups.

Nor are there any special affinities between the Insects and Sea-Spiders, but there is one striking similarity between the latter and Peripatus, which I have already spoken of; but an isolated fact of this kind gives little ground for further comparisons. The ventral organs in the two groups present a striking agreement, but there is no proof forthcoming as to a real homology of the structures. The process of the formation of the endoderm described by Heider and by Wheeler in Insects shows a certain resemblance to multipolar delamination, but if it be such it is a more complicated form than shown by the Pycnogonids. With these two exceptions there seems to be nothing else in common in the ontogeny.

We are then left to decide between an independent origin for the Pycnogonids or a relationship with the Arachnids. Prof.

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Dohrn has ably maintained the first theory, and the preceding translation gave the conclusion he had reached. Dr. Hoek likewise, as we have seen, holds this opinion, although not agreeing as to details with Prof. Dohrn. On the other hand, a study of the early stages of the embryology has brought to light certain facts which for me point decidedly toward a community of descent between Arachnids and Pycnogonids. The latter show undoubted traces of degeneration, and we cannot derive them from any existing animals. But I believe the Pycnogonids and the Arachnids have come down along the same line, or, in other words, have had ancestors in common long after those ancestors came from Annelid-like forefathers. The reasons for such a belief are as follows:

1. The Pycnogonids form the endoderm by a process of multipolar delamination, which is shown in its simplest form in Phoxichilidium and Tanystylum, and in a more modified condition in Pallene. In no other group of the Triploblastica do we find a similar phenomenon except in the Arachnids. The description of the embryology of Chelifer which Metchnikoff has given shows this process or something quite analogous to take place in it. The segmentation is holoblastic, and at a later stage the large cells containing yolk divide into an outer, more protoplasmic layer of cells and the inner cells, which are very granular. The outer form the ectoblast, and most probably the inner, judging from his figures, form the entoblast.

In the Spiders the process is not so well marked, but if the conception which Balfour had of the formation of the yolk-nuclei be true, then we may make a direct comparison between the two groups. He says: "It appears to me probable that at the time when the superficial layer of protoplasm is segmented off from the yolk below the nuclei undergo division, and that a *nucleus with surrounding protoplasm is left with each yolk column.*" This description for the Spiders may be substituted, word for word, for the process of delamination of Pallene.

2. The first trace of the embryo to appear in Pallene is a round opaque area at the spot where the stomodæum invaginates. In Schimkewitsch's recent account for the Spiders he shows that the primitive cumulus in them is the place where the stomodæum invaginates. This is also true for Pallene, but here

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the stomodæum invaginates quite early and perhaps simultaneously with the early formation of mesoblast at this place. Further, Schimkewitsch has called attention to the fact that the stomodæum of Spiders in its earliest development is a triangular invagination, and he compares it directly with the triangular invagination of the œsophagus of the Pycnogonids.

3. The early formation of body-cavity surrounded by mesoblast in the legs of Spiders has an exact parallel in Pallene and Phoxichilidium. Yet, however tempting such a comparison may be in this connection, it must be admitted I have not conclusively proved this to be true for the Pycnogonids, but only exceedingly probable.

4. In both Arachnids and Pycnogonids we have well-marked diverticula from the mid-gut into the legs. In the Pycnogonids these go into the cheliceræ and the four pairs of walking legs, and the same holds for the Spiders; but from a comparison of the appendages of the two groups we must suppose that the second pair of Pycnogonid's appendages to have lost their diverticula, and the last appendages either to have acquired diverticula, or more probably inherited them together with the appendage. In Chelifer the diverticula appear very early in development and contain some of the yolk from the mid-gut. This is shown very distinctly in Metchnikoff's figures for Chelifer, and in this respect the embryo resembles closely the embryos of Pycnogonids.

5. In both Arachnids and Pycnogonids the first pair of appendages are chelate. This in itself would draw attention to the similarities of the two groups, but we know further that in both groups this first pair of chelate appendages is innervated from the brain. These facts were considered by Balfour sufficiently important to indicate alone a relationship between the groups. He says: "The presence of chelate appendages innervated in the adult by the supra-œsophageal ganglia rather points to a common Phylum for the Pycnogonida and Arachnida, though, as shown above, all the appendages in the embryos of true Arachnida are innervated by post-oral ganglia."

I have not been able to find any post-oral ganglia for Pallene, but the first pair of appendages arises on the sides of the stomodæum and later moves forward. In this respect it compares closely with the Spiders, and the early innervation of this pair

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from the brain itself may be regarded as a more abbreviated condition than what was seen (by Balfour) in the Spiders. Metchnikoff's figures for Chelifer show the first pair of appendages to arise above and on each side of the proboscis-like upper lip. If future work verifies Metchnikoff's suggestion that this proboscis (rüsselformige, provisorische Oberlippe) is homologous, entirely or in part, to the proboscis of the Pycnogonids, as his figure might indicate, then does the whole development of Chelifer show remarkably close resemblances to the Pycnogonids.

6. The fourth pair of ambulatory legs—the seventh pair of appendages—has been a stumbling block in the way of those who have compared Pycnogonid with Arachnid. Semper and Schimkewitsch have attemped to solve the difficulty by assuming that the third pair of appendages of the Pycnogonids—the ovigerous legs—are new structures, and then have called the four pairs of walking legs homologous in the two groups. Prof. Dohrn has shown the impossibility of dropping out in the count the ovigerous legs, and has shown that this pair of appendages are homonomous with the others. The two pairs of ventral organs in the anterior larval ganglia we have seen point unmistakably to the same conclusion and give the final proof, if such were really necessary.

We are led then to a comparison of the appendages of the two groups, beginning with the cheliceræ and going back pair for pair, which leaves one pair over for the Pycnogonids. Any comparison between the two groups must take into account this extra pair of legs. Balfour has suggested that this last segment and its appendages to represent the first abdominal segment of the Arachnids. The third pair of appendages of the Pycnogonids have assumed a special function, and at this time we might suppose that an additional pair to have been added on from the abdominal segments. We also know that the embryos of Spiders have rudimentary appendages on the abdomen, and, as we have assumed the Pycnogonids to have come from the latter group, not recently but remotely, when these appendages may have been larger or even functional, we have some ground for a belief of such an origin of the last segment.

There is another fact which may be of importance in this connection. Not only is the seventh pair of ganglia carried dorsal-

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wards by the abdomen, but at the same time the sixth pair of ganglia also is carried dorsalwards and above the fifth pair, from which the connecting nerve fibers pass upwards to the sixth. At the present time it is impossible to determine whether this is due to a mechanical adjustment between the shifted abdomen and the last pair of thoracic ganglia, or whether the sixth pair, belonging properly to the abdomen, has taken part in the general shifting of that structure.[1]

There are certain objections against this comparison which I have attempted, and if they do not directly oppose yet do not support the hypothesis. First and most important is the uncertainty of brain invaginations in Pycnogonids. These seem to be present in all the Arachnids, and easily seen in the development of the embryo. In the Pycnogonids I have not been able to find such invaginations. We have seen in Fig. 19 that the groove might possibly bear such an interpretation; but even if this were true, we would expect to find a much more pronounced involution, but this does not seem to be the case. Again, the ventral organs, which have been compared with those of Peripatus, lend no support to the hypothesis. It is possible that the Pycnogonids have come from the Arachnids, at a time when these latter have had such organs in common with the ancestors of the Insects, and that they have been fully retained in the Pycnogonids. Lastly, the openings of the reproductive organs. Typically the ovaries and testes of the Sea-Spiders are a pair of organs united posteriorly by a cross commissure. They extend into the walking legs and open on the second joint of these. There are many exceptions to this, but they are regarded as secondary. The openings on the legs have no homologue in the Arachnids, except perhaps in Limulus, nor does it furnish any ground for comparison with other Arthropods. But if we assume the group to have come directly from the Annelids, we have no better ground here for a comparison. We are greatly in need of observations on the development of the sexual openings, and until we get such, the

[1] If we assume with Hatchek a common descent for all Arthropods, and that in the Insects we have several of the anterior segments about the mouth, suppressed, we might assume that the Spiders have lost a third pair of appendages, and the Pycnogonids retained it, and in this way bring into line the other appendages of the groups.

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question must remain an open one. It seems not improbable, however, that the openings may be secondary and connected in some way with the secondary presence of reproductive organs in the appendages.

I hope to have shown that these three objections are of negative value, at any rate so long as the present uncertainty surrounds them, and that we have sufficient grounds for a comparison in the early stages of development and in some of the important adult structures of the two groups.

The Pantopod-Larva.

There is a general resemblance between the Nauplius of the Crustacea and the larva of the Pycnogonids, but the differences become greater and greater the more closely we examine the two forms. In each the body contains three pairs of appendages, but those in the Nauplius show biramous structures, while none of the Pantopod-larval appendages show such structures. Moreover, the first pair of appendages of the Pantopod-larva is chelate and innervated from the brain. Other characteristics of the larva are the well-marked proboscis with its triangular œsophagus, the mid-gut sending out prolongations into some of the appendages, and the absence of any anal opening.

Have we then any basis for the assumption that this Pantopod-larva is a modified Trochophore of the Annelid ancestors? The problem is very similar to that of a supposed relationship between the Nauplius and the Trochophore, and in both cases an answer exceedingly difficult to give. Prof. Dohrn believes that the Pantopod-larva is to be regarded as a Trochophore with the Pycnogonid characteristics reflected back upon it. Prof. Lang believes in a similar process for the Nauplius. Without discussing the latter, let us confine our attention to the possibilities of the Pantopod-larva.

What characters have been "reflected" on Dohrn's theory, and what remains after these are removed? Prof. Dohrn has given us the answer. ". . . The absence of an anal opening, the pincers of the first pair of appendages, the long claws with the accessory spine, the structure of the proboscis with its triturating apparatus and its ganglia, the form of the tegumentary glands and their characteristic hairs, can only be considered as having been

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acquired in a much later stage and transmitted to larval life." That is to say, that almost every characteristic of the larva has been handed back from the adult! And what remains? "Nothing but the nervous system . . . an intestine, three pairs of appendages and two eyes." "But these are the attributes which one finds equally in the larva of Annelids!"

There is one vital fact which has been left out of account, viz. the presence of an anus in the Trochophore. How can we account for its absence on the above hypothesis when we know the Pantopod-larva to be a free-living larval form? And unless some special reason for such a loss can be imagined, the very basis of the comparison between Pantopod-larva and Trochophore is gone! What we have done in the above process of subtraction is to have removed the most striking structures of the adult from the larva and have left, not a Trochophore, but only the framework of the Pycnogonid.

For two main reasons I am unable to believe in the phylogeny given by Dohrn or by Hoek. First, because it seems to me there are facts derived from the early stages of development which point unmistakably to a relationship between Pycnogonids and Arachnids; and in the second place I cannot believe any actual homology to exist between the Pantopod-larva and the Trochophore, nor any fair reasons to assume that the characteristics of the Pycnogonids have been reflected upon a Trochophore.

If, then, we start with the assumption that there is a relationship between the Sea-Spiders and Arachnids, we may examine into the meaning of the larval form as a corollary to such a position.

I have stated that the Pycnogonids are degenerate, and probably not derivable directly from any existing group. If so, how far down in the ancestral tree of the Arachnids have they arisen? The very great differences in the adult structure of the groups indicate no very recent origin, but possibly they came in at a time when the Arachnids had the first pair of appendages chelate, and these were innervated from the supra-œsophageal ganglia, and had coeca from the digestive tract into most or all of the appendages. After the divergence of the Pycnogonids as a group from the general phylum of the Arachnids, the Pantopod-larva may have developed.

The Pycnogonids have adapted themselves to a very special

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habitat, and in general, what we may suppose to have happened, was a decrease in the size and an increase in the number of eggs, with the resulting early development of the free larva that we find to-day. This larva represents the more anterior segments of the adult, viz. that part containing the proboscis, the œsophagus, the eyes, the chelae, and two post-oral appendages. Behind this is an undeveloped part, which slowly grows in length as the animal increases in size. The anus belongs to the last segment, and does not appear until that segment is wholly or in part developed.

If the Arachnids have come from Annelid ancestors with many segments, we have a clue to the slight resemblance between the Pantopod and the Trochophore. The former represents the most anterior segments of the adult Sea-Spiders, and therefore to some extent the anterior segments of the Annelids or of the Trochophore. But at no time in the ontogeny of the Pycnogonids have Trochophore and Pantopod-larva been transformed the one into the other, as Dohrn believes.

Such seems to me the more probable view of the meaning of the Pantopod-larva. This belief has grown out of my work on the embryology of the group, and whether future work supports or disproves such an hypothesis, it is hoped it may be useful, if only as furnishing another point of view for looking at the phylogeny of the Pycnogonids, or may lead to a more complete study of the embryology of the group.

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PART II.—THE METAMORPHOSIS OF TANYSTYLUM.

The material for the second and third parts of the present account was collected during the summer of 1890, while at the Marine Biological Laboratory at Wood's Holl, Mass. I wish to express my gratitude to the Director, Prof. C. O. Whitman, and to the trustees of the laboratory, for the opportunity to carry on investigations under the favorable conditions offered at the marine station.

My object in giving the figures and description of the metamorphosis of Tanystylum is, in the first place, because I was fortunate enough to obtain probably a complete series of the larval stages of this Pycnogonid, and inasmuch as the remarkable condition of the development, and adult condition of the appendages of the Pycnogonids, offer in themselves a most interesting field for study, I hope a contribution to the complete metamorphosis of one form may be found useful. Again, by means of serial sections of each of these stages, I hope to have added a little to our knowledge of the internal changes taking place during development, as heretofore we have had only such knowledge as could be gained by surface views. Lastly, the figures give a series to which intelligible reference may be made when speaking in Part III of the Development of the Eye.

Not much difficulty was experienced in getting excellent sections of the embryo during its metamorphosis. As described in Part I, the embryos were killed in alcoholic picro-sulphuric solution (35 per cent alcohol saturated with picric acid, to which the usual amount of sulphuric acid was added). Before staining, the appendages were cut off (only possible, however, in the later stages) and the body stained overnight in hæmatoxylin; afterwards embedded in paraffine and cut into sections, which are better if cut so thick as 10 to 15 μ. The figures on Plate V were all drawn to scale, showing the enormous increase of growth during larval life. Figs. XXV and XXVI of Plate VI were also drawn to the same scale.

I cannot be sure that the series of figures drawn on Plate V and Figs. XIX, XX, XXI on Plate VI give the perfectly com-

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plete metamorphosis, although I believe the set must be almost filled out especially for the later stages. Here I could procure great numbers of individuals, and, comparing them and finding no intermediate stages, I inferred none such existed. But in the very young stages, before the appearance of the first pair of walking legs, it is extremely difficult to know how complete the series is; but from the slight gradations in size and structures in those figured, I think we shall not be far wrong in assuming an almost or entirely complete series.

In Part I of the present paper I have figured and described at length the structures of the Pantopod-larva of Tanystylum at the time when it leaves the parent. I shall speak of an embryo of this age as *stage* 1 (see Plate IV, Fig. IX). In examining the hydroids for Sea-Spiders, I found one embryo a little larger in size than this first stage, and having other slight differences which plainly show it to be a stage in advance of the last, and probably a stage immediately following *stage* 1, or after the first larval skin is shed. Figures of this *stage* (2) are seen in X and XI of Plate V, showing the ventral and dorsal views respectively. The embryo has much the same shape as *stage* 1, but there is seen an important difference between the two stages. The older larva has the posterior part of its body filled with a solid lobed mass of cells, which contrasts strongly with the clear posterior part of *stage* 1. Further, the second pair (really the third, as the first is double) of ventral ganglia are much larger. The posterior pair of lobes of the mid-gut project to the sides of the embryo, and an examination of the periphery of the posterior border shows a slight indentation on each side, indicating the beginning of another pair of appendages. The first pair of appendages, the mandibles, are three-jointed, and have, projecting forward from the first, proximal joint, a strong spine, as in *stage* 1. The second and third appendages are each two-jointed, with a strong terminal, movable spine. The proboscis lies in the mid-ventral line, and projects forward between and ventral to the first pair of appendages. Fig. XI shows the dorsal view of the same embryo. From the figure we see that the digestive tract of the embryo is on each side three-lobed. The first pair of lobes projects into the first pair of appendages, the second pair of pouches towards the base of the third pair of appendages, and the third

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pair of pouches projects into that portion of the posterior part of the embryo from which the next pair of appendages arises. In the anterior dorsal part of the larva are seen the first traces of a pair of eyes; the first pair of appendages is bent towards the ventral surface.

This embryo was stained and cut, and from the series of sections the following facts obtained: There is a small brain connected by means of a pair of commissures with the anterior ventral ganglion. This first ventral ganglion is, as we saw in the earlier larva (Part I), really a pair of fused ganglia. Behind this lies the second ganglion (the third of the series). The œsophagus, running back from the mouth through the proboscis, opens into the mid-gut. The latter has a pair of prolongations into the base of the first pair of appendages, and another pair of pouches run to the base of the third pair of appendages. The posterior part of the larva contains a backward continuation of the digestive tract, which seems still to end blindly behind. The cells lining the wall of the gut are now quite different from those observed in *stage* 1, which were seen in Fig. 13, Plate 1. In the present *stage* (2) they are much larger, are filled with large round masses of yolk-like substance, so that the lumen of the gut is almost obliterated. The yolk-like substance must have come from the exterior, and indicates that the animal is feeding at this stage and, perhaps, is supplied with abundance of food-material. No solid extraneous matter was ever seen in the tract of either larvæ or adults of Sea-Spiders, so that the food is probably obtained by sucking the juices of other animals—another similarity to Arachnids!—and probably from the hydroids, amongst which they live. The series of sections did not show any traces of a heart. A single pair of eyes was seen over the brain, and to the sides of these a thickened mass of ectodermal cells.

Passing to what seems to be the next stage of development, we have an embryo, shown by Fig. XII, Plate V, *stage* 3. There is quite an increase in size, especially marked in the length of the embryo. The first pair of appendages and the proboscis are longer and larger than in the last. The second and third pairs are as in the preceding stage; but the embryo now shows clearly external indications of a fourth pair of appendages. The preserved larva was quite opaque, and nothing of the

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internal organs could be made out from the surface, so that sections were again resorted to. Here it was found that the mid-gut gave off the usual diverticulæ to the first and third pairs of appendages and, besides, quite a large pair of pouches into the undeveloped fourth pair of appendages. The sections show that the gut ends blindly behind, and no trace of proctodæum was found. The lumen of the digestive tract is almost entirely obliterated by the large size of the cells, and these are completely filled with spherules of yolk. Large, active ectoblast and mesoblast cells, together with the mid-gut, fill up the posterior part of the embryo. The fourth pair of appendages is quite far advanced, as the ectoblast, by pushing in from behind, has partitioned off on each side a part of the posterior part of the body. The ingrowth of ectoderm on each side is always two-layered, furnishing a new wall to the outer sides of the body and to the inner side of each new leg. The first (double) pair of ventral ganglia is followed by a well-developed second pair, each containing large cavities, and these are followed by a smaller fourth pair of ganglia. There is a single pair of eyes.

The next embryo which I have found shows a considerable increase in size, and I cannot be certain that it is the stage following the last. Still it is probable, as I have found five or six of this size and none in between this and the last. The larva is shown by Figs. XIII and XIV giving the ventral and dorsal views of this *stage* (4th). The most marked external change is in the great elongation of the body posterior to the third pair of appendages, and in this region we can distinguish a central middle lobe and a pair of lobes on either side of this closely pressed against the middle part, the side lobes being the 'anlage' of the fourth pair of appendages. Three pairs of ventral ganglia were made out from surface views, but sections show also a small forming fourth pair. The embryo shown by Fig. XIV is the same, but from dorsal view. It shows a slight constriction in the middle of the body at the base of the fourth pair of appendages, and the continuation of the body posteriorly between these appendages is seen to be broader than the same region on the ventral surface; and further the base of the middle posterior lobe suddenly widens out, as seen in the figure. A single pair of eyes is shown on the anterior dorsal surface. Sections of this

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embryo give these points: The third pair of ganglia still have large cavities and a small fourth pair is in process of formation. The mid-gut sends the usual diverticulæ to the first and third pairs of appendages, also quite a large pair of pouches to the fourth appendages, and lastly a short pair, in the region of the third ganglia, runs out at right angles to the gut. These last pouches pass into that part of the embryo corresponding to the sudden widening at the base of the middle posterior lobe. Sections show that at this place the ectoblast pushes into the body cavity from behind forwards, forming partitions which are the beginnings of a new pair—the fifth pair—of appendages. Posteriorly the mid-gut seems to be continuous with a proctodæum which, I think, forms at this stage. It also seems probable that the heart forms at this stage of development, but the sections I have do not speak definitely on this point. I could not discover any trace, as yet, of reproductive organs.

We come now to a stage which, I suppose on account of its larger size, is easily found and not at all uncommon. I think it is undoubtedly the stage formed immediately after the moulting of *stage* 4. The small undeveloped fourth legs of *stage* 4 have now, in *stage* 5, Fig. XV, spread out laterally after the shedding of the cuticle, and have become segmented. The whole larva is longer than in the last stage, and all the external organs are also comparatively larger—the appendages, the proboscis, and especially the posterior part of the body. The most marked change is in the addition of the large fourth pair of appendages —the first pair of walking legs of the adult. Each of these is at this stage composed of *six* segments, of which the first three are somewhat smaller than the following four distal segments. The fourth segment of each limb, counting outwards from the body, shows internally traces of division into two parts, although the chitin covering the ectoblast is not divided. The spines projecting from the three distal segments of the legs must assist greatly in clinging to the hydroids, and particularly the long terminal hook with its two side hooks. Five pairs of ventral ganglia are seen from the surface—the first, as we know, being composed of two pairs. Other differences are easily gathered from the figure. Sections show that the digestive tract opens posteriorly to the exterior by means of a proctodæum. The first

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pair of diverticula from the gut runs forward into the limb as far as the glands of the mandibles, which are still retained.

Where these pouches join the digestive tract a second pair of diverticula originate and passes to the base of the third pair of appendages. A large pair of diverticula run out into the penultimate segment of the fourth pair of appendages, and behind these is a pair of pouches, the fourth, into the fifth pair of appendages, and, lastly, traces of a fifth pair of pouches is found. In Plate VIII, Fig. 52, there is a cross-section through the posterior part of this larva. It passes through the fifth pair of appendages, which are still at the sides of the body, and below the section passes through a pair of small forming ganglia. This section shows the thick ectoblast, the scattered mesoblast cells, especially abundant in the legs, and the large endodermal cells filled with food particles. On each side of the ventral ganglia are two other invaginations of the ectoderm. These push in both above (not seen in this section) and below and from behind —forwards as well, and form a partition wall in the body cavity, which cuts off a pair of appendages. The invagination for the ventral ganglia, shown in the figure on each side of the ventral median line, are the ingrowths for the fifth and last pair of body ganglia. There is a distinct proctodæum opening behind. In a single section of this series I found a well-marked 'anlage' of the sexual organs. It was on the dorsal surface of the digestive tract, in the middle line, and in that part of the body corresponding to the base of the first pair of walking legs. It was thickest in the middle, and tapered away towards the sides of the larva, but was confined entirely to the body. Dorsal to this mass of cells of the reproductive organ was what seemed to be an accumulation of blood corpuscles, and I fancied I could make out traces of a large heart cavity. In another embryo of the same age as the last I could find no traces of reproductive organs. There are four eyes on the dorsal surface, and the first pair are nearer together than the posterior pair, and on each side of the first pair there is a thickened mass of ectoblast cells. We saw there were five pairs of ventral ganglia, and it is worthy of note that the embryo with a single pair of walking legs has formed the 'anlage' of all the other ventral ganglia to the appendages.

In *stage* 6, represented by Fig. XVI, we find a second pair

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of walking legs, and, moreover, the embryo has increased a great deal in size. The first three pairs of appendages are essentially as in the last stage. The fourth pair has now acquired its adult number of segments, viz. eight. The two new segments which have been added were interposed, the one between the fifth and sixth (distal) of the preceding stage, and the other in the middle of the fourth pair, in which we saw faint internal traces of division in the last stage. The newly acquired fifth pair has now six segments, three smaller proximal ones and three distal.

In the posterior part of the animal the undeveloped sixth pair of appendages appears on each side of the body, and in between the bases of these appendages appear the small 'anlage' of a seventh pair. Five pairs of ventral ganglia are seen, the posterior larger than in the last stage. The posterior part of the abdomen lies at this stage still in the same plane as the rest of the body. The diverticula of the digestive tract are in the first appendages as in the last stage, the glands in the mandibles are still present, and the fifth pair of ventral ganglia still retain their invagination cavities.

The 'anlage' of the reproductive organs appears between the first and second pairs of walking legs in the mid-dorsal wall of the digestive tract, and is larger than in the last stage. In the anterior part of the body there seems to be formed a large heart cavity.

After the next moult (*stage* 7), Fig. XVII, we see an important change has taken place in the appendages. The third pair has almost completely disappeared, and only two small stumps indicate the point at which their appendage last appeared. The first and second pairs of appendages are unchanged in structure. I found one embryo of this age (7) having still the third pair of appendages, and for some reason the larva was quite appreciably larger than that given for this stage. Another pair of walking legs has been acquired—the third—and each of these has six segments.

The second pair of walking legs (the fifth pair of appendages) has now acquired its complete number of segments, eight, while the first pair retains its previous number of eight. The new segments which have appeared in the third pair of walking legs

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were interpolated at similar places to those of the preceding leg, viz. in the middle of the fourth and in between the fifth and sixth. There is an undeveloped pair of appendages at the posterior part of the larva, and the abdominal part of the body, instead of lying between them, as previously, has now moved dorsalwards, so that it projects from the posterior-dorsal part of the embryo. The first and second pairs of ventral ganglia (the first and second and the third in a true count) are in close contact or partially fused. The reproductive organ has much increased in size. In the region of the first pair of walking legs it extends through a few sections as two entirely separated parts, lying on each side of the middle line. In the region of the second pair of walking legs the two lateral parts unite into a single median structure which is almost as broad as the body, and the organ ends soon after this as a median, thickening on the mid-dorsal surface of the digestive tract. The glands of the mandibles have almost entirely disappeared.

At the next stage of development we have the formation of the seventh and last pair of appendages (*stage* 8), Fig. XVIII. There is a great increase in size, and an important change takes place in the second appendage. These lose their early larval structure and probably their first function, and now enter upon a new role. They are three-jointed and have lost the large terminal spine. The tip of the appendages is covered with many smaller spines, and sometimes I have seen one larger than the others, which presumably represents the large terminal claw of the preceding stage. The appendage is now three-jointed, having acquired an additional segment.

The first pair of appendages do not differ from the preceding stages. The third pair is still in an undeveloped condition, although somewhat larger than in the last stage. The fourth and fifth and sixth pairs have each eight segments, but the seventh pair, which has just been acquired, has only six segments. All of the walking legs have increased greatly in length, and each contains a long diverticulum from the digestive tract. Five pairs of ventral ganglia are seen, although the first and second are almost completely fused.

Sections show that the mid-gut sends out diverticula to the first, fourth, fifth, sixth, and seventh pairs of appendages and a

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pouch to the base of the rudimentary third pair. This pair of appendages is filled with embryonic mesoblast, and the ectoblast covering the appendage is thrown into a series of crenations beneath the cuticle, so that it would be much longer if extended, and is in condition to expand rapidly at the moulting period. This folding of the walls of the undeveloped appendage was seen also in all of the pairs of walking legs at the stage preceding their extension, and makes intelligible the great increase in length of the appendage when the next moult takes place. At the point of union of the gut-diverticulæ of the seventh appendage to the central digestive tube there is formed in the angle a small posterior pair of ventral ganglia. These were not seen in surface views, but lie above and behind the last pair of ganglia seen in the figure (XVIII), and belong to the rudimentary abdomen.

The 'anlage' of the reproductive organs is divided anteriorly into two parts, lying on each side of the dorsal side of the mid-gut, and run as far forwards as the base of the first pair of walking legs. Behind these divisions are united into a single mass in the region of the second pair of walking legs, and disappear at the anterior part of the third pair. There is a well-developed heart lying dorsal to these reproductive cells.

In the next stage we have a change which causes the larva to approximate much more nearly to the adult structure. The most important part of the change consists in the almost total loss of the first pair of appendages. See Figs. XIX and XX, Plate VI. Looking at the animal from the ventral surface we see no trace of this pair of appendages. Turning the animal over (XX) we find two projections at the anterior dorsal border where the first pair formerly arose. Each projection has two segments. A change has also taken place in the second pair, for these have now doubled the number of segments, and, except in size, have the adult structure. The third pair of appendages (XIX) has increased greatly in size, and now these form a pair of prominent projections on each side at the base of the proboscis. Each is bent almost at right angles back onto itself, and lies closely applied to the body in the space between the proboscis and the first pair of walking legs. Externally they do not show divisions into segments. The only point to note in the walking legs is that the fourth pair have now eight segments. The first three

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ganglia have completely telescoped, and this compound ganglia, it will be remembered, innervates the second, third and fourth pairs of appendages, as well as the proboscis. On the dorsal surface (XX) is to be seen the two pairs of eyes equally dividing the periphery of the cupola-like projection of the dorsal surface. Sections do not give any additional facts worthy of note.

The last stage of larval life, or rather the last stages showing external traces of immaturity, is shown by Fig. XXI, *stage* 10, and although after this the larva undoubtedly undergoes several moults, accompanied by increase in size before becoming sexually mature, it did not seem necessary to figure such intermediate stages. The only change which has taken place in this stage XXI is in the appearance of segmentation of the third pair of appendages, and the number of the segments of this pair is that of the adult. The second pair of appendages has also increased in length, reaching to the distal end of the proboscis. All of the walking legs have the adult number of segments.

Having followed the larva through all these changes of growth, it may not be out of place to say a few words about the adult structures. A figure of the adult Tanystylum from the dorsal surface is shown by Fig. XXII. The compactness of the body of this species is exceptional and has been carried to such an extent that the bases of the walking legs are almost in contact, so that they radiate in all directions (except anteriorly) as from a central point. In most other Pycnogonids there is not so great a concentration of the body, and these undoubtedly come nearer to the ancestral form, and, moreover, in some of these simpler forms the rudimentary abdomen lies in the same plane as the body and behind the attachment of the posterior pair of legs. The figure for the anterior region of Phoxichilidium (Fig. XXIV) shows a form in which there is no such concentration. In Tanystylum, as seen in Fig. XXII, the abdomen has shifted far up on the back of the animal, and is separated by only a short space from the base of the prominence bearing the eyes. The adult still retains the rudiments of the first pair of appendages. These have a large basal segment and a smaller distal one covered with spines. Both males and females develop the ovigerous legs, and the males carry during the breeding season from one to three bunches of eggs on each of these appendages, as shown in Fig. XXIII.

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(This is not drawn to the same scale as XIV.) The bunches on each leg are entirely separated from each other, while in Phoxichilidium (see Fig. XXIV) the bunches are larger than in the last (but with the eggs individually smaller), and are stuck to both of the ovigerous legs, which are thus bound firmly together by the sticky masses of eggs.

Before leaving the metamorphosis of Tanystylum, I wish to refer briefly to the development of the ventral ganglia. I had previously shown in Pallene that each ventral ganglion was formed by an invagination of the ectoblast, and I was led to infer a similar formation in Tanystylum, and by inference for all of the Pycnogonids. A study of the metamorphosis of Tanystylum has verified this beyond all doubt for that form, and I think it will be legitimate to extend this to the whole group.

The earliest stages of the development of the ventral organs in Tanystylum are shown in Fig. 52, Plate VIII, where the thickened ectoblast pushes in at two points on each side of the mid-ventral line. The nuclei of the cells of the invagination at this stage are scarcely, if at all, larger than those in the rest of the ventral ectoblast. A later stage in the formation of these ventral organs is shown by Fig. 54. It is taken from the third pair of ganglia. In this the cavities of invagination have closed, and the ganglion is now cut off from the ventral ectoblast. There are many ganglion-cells or nuclei forming the ganglion, and the nuclei in the cells bounding the cavities are larger, and oftener show karyokinetic figures than do the other nuclei of the ganglion. The last stage of development is shown by Fig. 53. Here the central cavity is almost obliterated on the right side, but on the left side the section cuts a little to one side of the central cavity, and only the cells of the wall appear, and two of these are seen in process of karyokinetic division. It is to be noted that the nuclei of the ventral organs at this stage are seen to approximate in size to those nuclei forming the substance of the ganglion, but stain more deeply. If we examine a little later stage, the central cavities of the ganglia have disappeared completely, the nuclei are all of the same size, and the only indication of the ventral organs is in the deeper staining of the cluster of nuclei at the spot in each ganglion where the cavity was last seen. Ultimately this difference also disappears.

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In the first part of this paper I was rather inclined to believe that the ventral organs had a phylogenetic significance, but still I must believe their interpretation to be an open question. The large size of the nuclei of these organs during their time of greatest activity in forming, and the subsequent merging of these nuclei (and cells) into ganglion nuclei (and cells), suggest that there may be no ancestral meaning to the process, but that there may be larval organs—something like neuroblasts—for the formation of ganglia. And it has been suggested to me that these may fall into the same category as the neuroblasts of Annelids, and may present, by analogy, an intermediate step in the formation of such cell-masses or teloblasts.

Another interesting problem might be discussed if there were space, viz. the appearance and subsequent loss of the third pair of appendages; and, again, the curious law discovered in the sequence of the walking legs, and its relation to the number of segments. When a pair appears for the first time, the number of segments is six; at the next moult the number increases to eight, by the interpolation of one and the division of one into two, and this method of increase is common to all of these appendages.

In thinking, too, over the remarkable series formed by the different species of Sea-Spiders, where, as Dohrn has shown, we can proceed from the simplest to the most complex, finding amongst living forms all intermediate stages, one finds a most suggestive field for speculation. Prof. E. B. Wilson has pointed out to me the great perfection of this series, and I have since been interested to find several *other parallel cases in semi-parasitic groups* of animals, as, for instance, in the Copepods, in the Cuninas, in the Barnacles and in the Sponges. It would be interesting to discover the correlation, if any, between the persistence of a perfect series of animals of a group and semi-parasitism.

[There are two other figures, XXV and XXVI, which I have added to Plate VI, but these are not directly connected with the second part of this paper; nevertheless, as I only obtained them after the figures to the first part were printed, I have determined to add them now for the sake of completeness. The first figure is of the larva of Phoxichilidium at a time when it leaves the

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parent. This larval form shows certain differences from that of Tanystylum. In it the second and third pairs of legs are much longer, as the distal spine is prolonged out into a long thread-like continuation of the limb. In Tanystylum these spines were thick and short and adapted for crawling amongst the hydroids, while the long limbs of the larva of Phoxichilidium could hardly be used in this way, and suggest rather that they are used for catching to any objects they may come in contact with. The first pair of appendages are thicker in Phoxichilidium, with smaller claws, and the proboscis, instead of projecting forward between the first appendages, is only raised slightly above the level of the body at about its middle point. I have not obtained later stages of this larva, but the accounts of other species of this genus show that it becomes parasitic either inside the digestive tract of a hydroid or buried in its substance, and here it undergoes its metamorphosis until the adult structures are almost reached.

The other figure (XXVI) is of a young Pallene after it has left the parent, and this individual was found living freely amongst the Pennaria. The figure shows a ventral view, and the stage corresponds to one following that shown by Figs. VII and VIII of Plate IV. It shows the third pair of appendages beginning to develop, which, in Figs. VII and VIII of Plate IV, only appeared as two slight projections from the sides of the body. In the present case these appendages are folded across the breast—if the term be applicable to Pycnogonids—closely adhering to it in the region between the base of the proboscis and the first pair of walking legs. The embryo has also fully developed its fourth pair of walking legs, which, it will be remembered, were in an undeveloped state in the previous stage.]

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PART III.—THE STRUCTURE AND DEVELOPMENT OF THE EYES OF PYCNOGONIDS.

In reaching a decision as to the systematic position of the Pycnogonids, I hoped to test the conclusions I had reached from a study of the embryology by taking another point of view. Fortunately a most interesting field opened in a consideration of the structure and development of the eyes. While enjoying the opportunities of the Marine Biological Laboratory I was enabled to procure and study an abundance of fresh material. The advantages of using such material is now so well known as to need only mention, and I am certain that I could not have obtained nearly so satisfactory results from preserved animals.

The results obtained from a study of the eyes have added, I believe, very much to the solution of the problem I had before me. Hoek and Dohrn give the only figures of the Pycnogonid eyes that I have found, but the results are confessedly incomplete, due in the one case to poor preservation of material, and in the other to the difficulties of technique. Neither of these accounts will be of any service to us in our study of the eyes, and may be dismissed here. The subject will be treated in the following order:

1. The Anatomy of the Adult Eyes.
2. The Histology of the Adult Eyes.
3. The Development of the Eyes.
4. Comparison with the Arachnid Simple Eyes.
5. Inversion of the Pycnogonid Eye.
6. General Considerations of the Arachnid Eye.

1. The Anatomy of the Adult Eyes.

The Sea-Spiders have in all four simple eyes, situated in the middle of the first (compound) segment of the body, on a raised, cupola-like projection. In some of the species the eyes are certainly degenerate, but in none are all traces of the eyes lost. The position of the eyes on the body can be best shown by reference to the figures. In Fig. XXII we see the dorsal surface of Tanystylum, and on this, in the so-called first segment, raised

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on a dorsal projection, lie the four eyes equally dividing the circumference. From the exterior each eye is seen surrounded by pigment, generally of a yellowish-black color. The eyes are extremely small and covered by thick chitin, rendering it exceedingly difficult to get at the eyes either for sections or for maceration.

Fig. XXIV shows a side view of the anterior end of Phoxichilidium. It shows the shape of the cupola-like projection bearing the eyes, two of which are seen on side view. The eyes of this species are much larger than the eyes of either of the other two Sea-Spiders, and for this reason were less difficult to manage, and the following account will apply to the eyes of Phoxichilidium, unless otherwise stated. "Alcoholic picro-sulphuric" solution gave by far the most satisfactory results. The body was cut across near to the eyes, and the portion containing the eyes fell immediately into the killing fluid. After thoroughly washing out the picric acid the eyes were stained in hæmatoxylin and afterwards cut in paraffine.

We may start with a figure showing the whole eye removed from the body, as seen in Fig. 30, Plate VII. The figure is of an eye which had been sufficiently macerated to free it from the surrounding tissue, removed in glycerine to a slide, and rolled around until the innermost part of the eye was turned upwards. The figure therefore shows the inner surface of the eye—that part nearest to the center of the cupola.

The pigment was, in handling, to some extent removed from this part of the surface, so that by focusing beneath this another layer of the eye could be seen. The figure shows the distribution of the optic nerve to the eye, which comes directly from the brain beneath the eye. The most important part of this figure is the arrangement of the parts of the eye on each side of a median line—the raphe. This runs through almost the whole inner length of the eye as a somewhat irregular line from above downwards, and is formed by the elements of the visual part of the eye coming in contact from the two sides. Indeed, in this figure almost seventy-five pairs of these elements are seen arranged along each side of the raphe.

The optic nerve, before it reaches the eye, breaks up into several branches, and the branches enter the eye along its inner

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surface and along a line corresponding approximately to that of the raphe. The nerve branches disappear in the pigment covering the inner surface of the eye, and cannot be followed farther.

If we make a section in a horizontal plane through the cupola, so that all of the eyes are cut transversely to their longest diameter which runs from above downwards, we obtain a figure shown in 31. The four eyes are about equally distant from each other. In front of each the chitin is greatly thickened to form a lens; beneath this the corneal hypodermis is very much thickened; under the corneal hypodermis is a cup-shaped mass composed of two layers—1st, a light layer containing nuclei, and 2d, a layer of pigment surrounding the first. The entrance of the nerve is shown in some of the eyes at their inner, middle part. If we examine one of these eyes under a higher power of the microscope, we obtain such a figure as is shown in Fig. 32. The chitinous lens is well shown here, and the great increase of its thickness over the eye is very marked. The ectoderm of the body, the so-called hypodermis, also undergoes a great change in the region of the eye. The cells are much longer, have conspicuously large nuclei, and tapering away at their inner ends into fine processes which run out to the two sides of the eye. The whole arrangement of these corneal-hypodermal cells—lentigen cells—is markedly bilateral, as seen in Fig. 32.

Immediately under the lentigen cells lies the middle layer of the eye. This is the retinal part of the eye, or that part sensitive to light. Immediately under the corneal hypodermis this middle layer of the eye shows a meshwork of fiber-like processes, which are probably interlacing and anastomosing nerve fibrils. Passing inwardly, there next follows a zone containing conspicuous nuclei and large vacuolated spaces. There next comes a zone which contains slightly stainable, rounded bodies slightly smaller than the nuclei. Lastly, in the bottom of this cup-shaped middle layer is a portion of the middle layer which stains more deeply than the rest of the middle layer, and radiating through this from the bottom of the cup outwards are several deeply stained (drawn black in the figure) rod-like bodies or bacilli. Corresponding somewhat to the intervals between the bacilli, the middle layer is indistinctly split up into

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bands running from without in, through what has been spoken of as the zones. These zones of the middle layer are not of course separated from each other, but pass insensibly into each other, and are only of use in describing the structure of the middle layer.

The nuclei found in the middle layer lie largely to the right and the left of the middle line of the eye, and through quite a wider zone of this layer. Thus they show to a large extent a bilateral arrangement. The inner layer is surrounded on its entire inner surface by a pigmented layer—the inner and pigmented layer of the eye. This layer is composed of pigment granules, both black and yellow, and it is impossible in such sections to resolve the layer into its constituent cells. The entrance of a part of the optic nerve is seen in the figure. It disappears in the pigment layer in the inner middle line, and cannot be traced farther, in sections, than the pigment layer.

Fig. 33 is a drawing of a longitudinal section through the same eye. It is therefore at right angles to the last figure and along the line of the raphe. This section, however, is not median through all its length, but at the outer part passes to one side of this line; at the inner part, however, it cuts approximately the middle line. But this slight obliquity is really advantageous to an understanding of the eye, as it passes more in the plane which the principal retinal elements follow. The three layers of the eye are clearly seen, although the distinction between the outer and middle layers is not so definitely marked as we saw in the cross-section, and is due here to the oblique plane of the section. Under the corneal hypodermis lies the large middle layer, in which we see the same structures as shown in the cross-section of the eye. The nuclei are conspicuous and numerous, and much more abundant here than in a section passing through the exact middle line. There are large vacuoles lying amongst the nuclei, but I have been unable to determine whether these are artefacts or natural to the eye. But I can scarcely believe them to be artificial products, as they seem to be constantly found on this part of the eye, and show no evidence of being due to tearing of the sections.

The layer of rounded bodies is not shown in the figure. The form of the retinal elements is better seen in this section than in the cross-section. At the inner part of the middle layer is a

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series of deeply stained rods—the bacilli, which occur regularly from the top to the bottom of the eye, and it was these which we saw in Fig. 30 arranged along each side of the middle line. These bacilli are continuous with the cell-walls of the retinal cells, which pass outwardly from the bacilli as far as the zone of nuclei. The third and inner layer of the eye covers completely the middle layer as far forward as the cells of the corneal hypodermis, but its ending in the region of the hypodermis was not well shown in the section from which this figure was taken. This inner layer is composed of variously shaped cells containing much dark pigment, but the shapes of these cells can only be determined by maceration.

It may be well to explain here that the cells composing the middle layer correspond in number to the bacilli and cell boundaries shown in this figure, and that they form a series of inverted retinal elements, the length of which is almost as great as the depth of the middle layer; and, as we shall see later, there is a nucleus to each of these elements.

There remains a third plane in which I have cut sections of the eye, viz. that passing at right angles to the length of the retinal rods. A few sections of this series are shown in Figs. 34, 35 and 36. The first of these (34) is the second section from the inside, the first section having cut off a slice of pigment. We see the periphery surrounded by pigment, and in the center we see the arrangement of the outer ends of the retinal cells where they come in contact from the two sides. The raphe is here clearly seen to be formed by the contact of the bacilli of the two sides of the eye, and is formed as a zig-zag line by the interlocking of the bacillar ends of the retinal cells. Between the bacilli the granular protoplasm of the cell is seen, which runs almost to the tip, so that the bacilli *are not formed at the inner ends of the cells, but along the side walls at the inner end.* It is also not to be overlooked that only the superficial layer of retinal elements interlock at the surface, forming the raphe; and, while there are many other elements in the interior of the eye, these do not seem to come to the inner surface, but lie just beneath these superficial cells. At one point in Fig. 34, viz. in the lower left-hand portion, the section passes a little below the superficial bacilli, bringing to light the transversely cut ends of two of the interior retinal elements.

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A section of the eye somewhat deeper—that is, more outwardly —than the last is shown in Fig. 35. Along the middle line the central elements are cut transversely, showing the size in cross-section of the outer ends of these elements. On account of the bending of the outer retinal cells around the surface of the eye, they are cut now farther down than in Fig. 34, where their walls are thinner. Other elements lying between the central and superficial ones are cut somewhat obliquely, and the walls of some of these are seen to thicken, and to join the bacilli as they approach the middle line. In the lower part of the figure one sees that the bacilli show a double structure, so that one-half of the thickness belongs to one cell and the other half to the neighboring one; so that they are really double, but usually the separation into two parts is not seen, owing to the close fusion between the cells. This figure also shows the entrance of a part of the optic nerve, disappearing in the pigment layer.

Fig. 36 passes almost through the middle of the eye, but probably somewhat nearer to its inner half. The most interesting structures shown in the figure are the small rounded stainable bodies in the central elements. These lie in the outer ends of the cells found in the middle of the section. Around these the protoplasm seems absent or thinner, so that each has sometimes a halo about it. In other cells they are not seen, although they show a clear portion in the protoplasm, and probably the bodies are cut at a different level. The interlacing of the cells themselves in the central part of the eye should be noticed. They are longer from side to side, giving them in cross-section a lanceolate shape. At the sides of the section lie many scattered nuclei. Lastly, the nerve fiber is seen entering the eye along the middle line. The sections following this one as we pass to the outer part of the eye add little or nothing to what we have gained from the previous figures, and did not seem worth drawing. The vacuoles in the outer part of the inner layer are very conspicuous in some of these sections, and one also finds a few nuclei almost, or quite, in the middle line, but at all times they show a marked tendency to bilateral arrangement.

Such is the gross anatomy of the eye as gathered from sections, and a great many sections were made, prepared by many different methods, to obtain even this meagre information. As a

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check to the results obtained in Phoxichilidium, the less promising eyes of Pallene and Tanystylum were studied. It is not necessary for our purposes to give a full account of the structure of the eyes of these forms, as they showed in all essential points a close agreement with Phoxichilidium. To call attention to a few of the differences may not be out of place, so I have given two longitudinal sections of the eye of Pallene and Tanystylum.

Fig. 37 is a longitudinal section through the eye of Pallene empusa. The most noticeable difference, when compared with the eye of Phoxichilidium, is in the absence here of any well-marked lens. The chitin is somewhat thicker over the eye than elsewhere, but not sufficiently so to form a definite lens. The three layers of the eye are clearly seen. The corneal hypodermis is not so well developed as in Phoxichilidium, and perhaps this is correlated with the non-development of the lens. The middle layer is essentially similar to that of Phoxichilidium, with its nuclei, vacuoles and rods. The bacilli are, however, relatively more abundant and closer together, and each bacillus is relatively longer. The inner pigmented layer is less well developed and forms a narrow yellowish zone around the inner part of the eye. Cross-sections add nothing to our knowledge, and sections at right angles to the raphe show that it, the raphe, is essentially as in Phoxichilidium.

In Tanystylum we find the same structures seen in the other two as shown in Fig. 38. The chitin over the eye is thicker than elsewhere, and forms a slightly developed lens, being intermediate in structure between Pallene and Phoxichilidium. The corneal hypodermis is much as in Pallene. The outer part of the middle layer shows few nuclei, which indicates it is near the median plane of the eye. The bacilli are fewer than either those of Pallene or Phoxichilidium. Fig. 39 is a part of a section across the raphe, and, passing through it, shows the arrangement of the cells on the two sides. It was impossible to trace in these eyes the nerve fibers after they disappear in the pigment layer. They seem, however, to run around the sides of the eye, probably innervating the pigment cells in their course, and enter the middle layer from the sides and at its outer part beneath the corneal hypodermis.

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Histology of the Eye.

The following account of the histology is confined almost entirely to a description of the middle and inner layer of the eye of Phoxichilidium. The shape of the cells of the outer layer is sufficiently shown in Fig. 32. They are seen to be columnar, and have conspicuous nuclei in the middle part of the cell. The cells themselves show a bilateral arrangement, the smallest being on each side of the middle line and thence towards the sides, increase in length and breadth for a time, when they again begin to get smaller, passing into the ectoderm of the body at the point where the pigmented layer touches the surface.

To obtain a knowledge of the middle and inner layers careful macerations were necessary, and as the results obtained by the different methods varied with the reagents used, it may be better to mention those found most useful. The easiest method to break up the eye into its constituent elements is maceration in a modification of Haller's fluid. The following proportions were used: Acetic acid (glacial) 5 parts, water 10 parts, glycerine 5 parts. The cupola bearing the eyes was cut off and quickly immersed in the fluid, remaining there from a half to an hour, washed in water, and teased out sometimes in water, sometimes in glycerine. The method has one disadvantage in that it causes the bacilli to swell up and become transparent, so that one may be misled—as I was at first—by the apparent absence of bacilli at the ends of the cells. The method has the great advantage in that it separates the individual elements completely from one another without breaking them up into pieces. Figs. 40, 41, 42, 44 and 45 were obtained in this way. A less successful reagent was that of sulphuric acid and sea-water. It has the advantage that the bacilli do not change their shape, but it does not easily separate the cells. Osmic acid was not very successful, probably on account of the difficulty of penetration.

After the macerated pieces were put under a cover-slip a little gentian violet was run under, which stained the cells and the nuclei, rendering them easier to study. If one is successful the middle layer breaks up into a great number of elements like those shown in Figs. 40, 41 and 42. Each of these elements—cells—is a somewhat flattened body with one clear end—the inner—and a more protoplasmic middle portion containing a nucleus

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which stains deeply with gentian violet (40). Beyond this there is a slenderer region which continues outwardly (upwards in the figure) into a protoplasmic syncytium. This syncytium lies on the outer part of the middle layer, under the corneal hypodermis. The cell thus obtained shows no trace of the bacilli, but their absence is due to the macerating fluid, which causes them to swell at the sides of the cell.

Fig. 40 *b* was the most successful maceration obtained. Parts of three cells—retinal elements—are seen each passing at their outer ends into a common syncytial mass, as in the former case. The upper part of the figure shows a fine fibril running also into the fused protoplasmic part, and this fiber could be traced outwards into one of the nerve branches running to the eye, and undoubtedly represents the ultimate nerve fibril which passes to the retinal elements. The distribution of the nerve to the outer part of the middle layer, and the bacillus present at the inner ends of the retinal elements, show conclusively that the retina is "*inverted*"; so that light entering the eye must, before it can penetrate to the sensory end of the retinal elements—the bacillar end—pass through the layer of nerve fibrils and fused protoplasmic layer of the eye.

Fig. 41 *a* and *b* shows to what an extent some, if not all, of the retinal elements are flattened. In *a* the spatula-like end of the element is seen, and this shape is due to the swelling of the bacilli at the sides of the retinal cell. In *b* the same element is shown as rolled over on its side, and is seen to be much narrower in this diameter than in the former. This accords fairly well with the shape of the elements seen in the section of Fig. 36, Plate VII.

In Fig. 42 three of the retinal elements are seen still in contact—two lying above a third, the inner end of which shows through the transparent ends of the others. Two have spatula-shaped ends and the third is for most of its length broader than the others. Each has a nucleus nearer the outer half where the protoplasm swells out the cell, and beyond this the elements taper away to join, no doubt, in the syncytium at the outer part of the eye.

In Fig. 43 is a portion of the inner end of a retinal cell which was obtained by sulphuric acid and sea-water, and the figure is

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more highly magnified than in the preceding cases. The protoplasm runs down to the end of the cell, but on each side are the rod-like bodies. On the left side of this element is a piece of a rod of a neighboring cell; the bacilli being formed by the fusion of a pair of these rods of adjoining cells. Further, the end of the retinal element has been so twisted that one also gets a side view of the rod, showing it to be thinner through than broad. This preparation is unsatisfactory, in that it is broken off at the point where the cell-wall is continuous with the bacillus.

In Fig. 44 we see a copy of a part of the superficially lying retinal elements of the eye of Pallene. It shows how the cells lie side by side in the superficial part of the middle layer, but at one point one of the under cells comes out to the surface. The nuclei are placed in the upper (outer) part of each cell, and below these cells abut against those on the other side and form the raphe (not seen here). All of the middle layer cells seem to be very much alike, and although I have examined quite a number of macerations, I have never obtained any decided departure from those given above. On the other hand, I cannot deny that other cells may exist in this layer, for, on account of the small size of the eyes and the minute size of the retinal cells, it would be very easy to overlook any unimportant elements, but I think there can be no doubt but that what we have called the retinal elements form the greatest part of the middle layer.

If we next examine the cells of the inner layer—the pigment cells—we find them showing a very great diversity of shape. They are easily macerated apart by Haller's fluid (modified as above). A few of these cells are shown in Figs. 45 *a*, *b*, *c*, *d*, illustrating the main varieties.

There seems to be a more or less definite distribution for these different kinds of cells, and we may refer them, I believe, to different parts of the posterior layer. Those pigment cells in the innermost part of the eye, lying over and at the sides of the raphe, are shown in Fig. 45 *a*. These are short, somewhat cylindrical cells, about as high as wide, and fitting tightly into one another. Ten of them form the mass in this figure, and in some of them the nucleus can be seen, and in all the cell boundaries made out. The cells found over the sides of the eye show great differences in size and shape, as seen in Fig. 45 *b*, *c* and *d*.

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In *d* the cells are drawn out at one side, becoming longer in one diameter, and this process seems continuous into a nerve fibril, though the process could only be traced for a short distance, and I have not succeeded in tracing it actually into such a fibril. In *c* two cells are shown which have fused together at the base, and from the point of union a process runs out which presumably is continuous into a nerve fibril. I think one must be impressed by the close similarity between two such cells shown in *c* and the similarly arranged retinal cells of Fig. 40. Now, although I have no actual proof that the cells *d* lie nearer to the cells *a*, and that the cells *c* are farther around to the sides of the eye, yet from what I have seen I think it exceedingly probable that in passing from the innermost cells of the pigment layer to the outermost (those nearest the hypodermis) we go through a series of changes as shown by *a*, *d*, *c*. Finally, in Fig. 45, *b*, there is seen a long, slightly pigmented cell resembling much in shape a retinal element. I cannot affirm positively that this cell belongs to the pigment layer, but it seems exceedingly probable that it does. If so we find quite a good series connecting the most modified pigment cell on the one hand with the retinal elements on the other; and, further, each of those pigment cells seems to connect by quite a large process with a nerve fibril. The important bearing of this arrangement, etc., will be discussed in another place.

The Development of the Eyes of Tanystylum.

Instead of beginning with the earliest stages of the eye, which are the most difficult to understand clearly, let us start at the other end, when the eye has not quite reached maturity, and work thence backwards.

Fig. 51, Plate VIII, is from a longitudinal section through the posterior eye of a larva which has just acquired four pairs of walking legs. (See Fig. XVIII, Plate V.) In this the ectoderm has, in hardening, separated somewhat from its chitinous covering, but there is no special thickening of the chitin over the eye to form a lens. The three layers of the eye which were found in the adult are here easily recognized. The outer layer, which is formed but slightly modified hypodermal cells, contains large nuclei, which are arranged in a single layer. The

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arrangement of the elements of the middle layer is not well shown in the figure, but other sections show that they are essentially as in the adult, and that small rods, bacilli, are developed along the sides of the inner ends. The eye also shows in other sections a distinct raphe. The inner layer is not so much pigmented as in the adult. The essential interest of this figure lies in the relationship it reveals between the first, the outer, and the second, the middle, layers of the eye. If we follow from above downwards the layer of corneal hypodermis, we find that when it reaches the lower angle of the eye it suddenly turns inwards and becomes directly continuous into the middle layer of the eye, and that the large nuclei of the outer can be traced continuously into the nuclei of the middle layer at this point.

This figure does not show the relationship between the pigment layer and the hypodermis, but others do, and it may be stated that just below the eye the pigment layer becomes continuous into the hypodermis, and at the upper part of the eye, as seen in this figure, the middle and inner layers are in close contact. We may interpret this condition of the eye by referring it to an invagination of the ectoderm, the opening, if it exists, being at the lower corner of the middle layer; that the two walls of the invagination are of different thicknesses, etc., the middle layer being one and the pigment layer the other; and, finally, the whole invagination being pushed over to one side —dorsalwards. I think this interpretation of the conditions is not forced, and is the explanation of the conditions shown not only by this but by other figures.

Figs. 49 and 50 are from a larva having three pairs of walking legs. (See Fig. XVII, Plate V.) These two figures belong to the same series, and are neighboring sections of one of the posterior eyes. The animal is about ready to moult, so that we find two distinct chitinous coats, the one closely adhering the other over the latter and not directly connected to it. Fig. 49 is somewhat to one side of the middle line, especially in the upper part of the eye, but near to it in the lower part. The corneal-hypodermal cells at the lower corner of the eye pass into the cells of the middle layer. The outlines of the cells are better seen than in the previous section, and small rod-like bodies are seen at their inner ends. The middle layer is smaller than in the pre-

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ceding older stage. A narrow zone of pigment cells forms the inner layer of the eye, and at the lower corner of the eye it passes directly into the hypodermis below the eye, while at the upper corner of the eye the pigment layer is never found intimately connected with the hypodermis, but is in close contact with the middle layer of the eye. Fig. 50 cuts the upper part of the eye in the middle line, but not so much so the lower. In the upper corner of the middle layer there lies a cell which is shorter and broader than the retinal cells farther down, and very often the second cell from the top shows a somewhat similar difference in shape. In fact, these two cells seem to be present in all the larval eyes, and the meaning of this we shall see later.

Fig. 48 shows the posterior eye of a still younger larva. (See Fig. XVI, Plate V.) Here again we find practically the same conditions as in the preceding figures, but the eye is smaller. The corneal hypodermis passes at the lower corner into the middle layer of the eye. The pigment zone behind the eye is narrow, and the third layer is seen to continue into the hypodermis below the eye. At this place the cells of the hypodermis are very distinctly seen passing into the pigment layer of the eye. The middle layer is separated very distinctly from the corneal hypodermis by an elongated cavity, probably caused by shrinkage.

Between this eye and the next earlier stage there is quite a difference in size and structure. This next stage is shown in Fig. 47, which is from a larva having a single pair of walking legs. (See Fig. XV, Plate V.) Both posterior eyes are shown in this figure (47). They are not, as in all older eyes, raised above the general surface of the body. Each eye is still seen to be three-layered, though this is not so apparent as in older stages. Both the outer and the inner layers are quite narrow. The middle layer is seen to be formed of two quite large clear cells with very large nuclei. These form by far the most conspicuous part of the eye in cross-section, but I have not determined definitely whether there are only these two cells, as seen in cross-section, or whether there are a few more present. There is, it must be confessed, not much evidence of invagination of the eye at this stage, and for this reason it seemed better to begin with older stages, which could be easily interpreted as such. How-

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ever, in the eye on the left side of the figure (47), the continuation of the corneal hypodermis with the middle layer is seen, and in the right eye the pigment layer is intimately connected with the hypodermis on the outer side of the eye; so that the arrangement is essentially as in older stages. I could not determine definitely as to the number of cells forming the pigment layer, but in cross-section one can discover not more than two nuclei, so that the number must be exceedingly small. Besides the pair of eyes seen in this figure, there is an anterior pair resembling the above both in structure and size. There is one difference shown in the sections of the anterior eyes, viz. that there is a thickening of hypodermal cells on each side just beyond the outer corner of the eyes, but of this I shall speak later. Moreover, the anterior eyes are nearer together than the posterior pair.

Fig. 46 is from a larva without any walking legs, at an age shown by Fig. XIV, Plate V. The larva has but a single pair of eyes, which correspond to the anterior of the two pairs of older larvæ. We find the eyes have essentially the same structure as in the one last described, but are much nearer together than those of the last figure (a posterior pair). Each is composed of three layers. Of these the middle is much larger, and is composed of two large transparent cells with large nuclei. The continuity of the corneal hypodermis with the middle layer was not clearly made out, nor that of the pigment layer with the ectoderm below the eye; but I think we may fairly push our interpretation even to this, but on account of the extreme smallness of the eye and the difficulty of making very thin sections, such connections would not be readily discovered. The posterior layer of the eye seems to be formed of but a single large flattened, pigmented cell, showing a clear nucleus, but it cannot be affirmed with anything like certainty that this is the only cell.

On each side of the anterior eyes the ectoderm, as shown in Fig. 46, is a greatly thickened mass, indeed forming two bodies about the size of the two eyes themselves. I am at a loss to interpret these thickenings. At first I believed they might be connected with the sudden increase in the size of the eye, and the very great change in size in the eye between stages XV and XVI of Plate V might seem to support such an hypothesis. But, on the other hand, the posterior eyes also increase greatly in size at the same

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time, and these have the ectoblast very slightly thicker at the edge of the eye. Possibly these may represent rudimentary eyes which were placed on each side of the first pair, or else have shifted position from some other place; at any rate, they are of interest and very constant in position, and furnish very little ground for speculation.

The structure of the eye of the Pantopod-larva was described in Part I. This description is correct, I believe, as far as it goes, but at the time I had little knowledge of the adult eye, and hence overlooked an extremely narrow layer of ectoblast between the two large cells and the chitin. I have since re-examined the eye of this larva, and can assert that in some sections this layer could be made out, and in others a thin membrane-like body separated the clear cells (middle layer) from the chitin, and this, presumably, is the same ectoblast. Unfortunately, the section from which the figure of Plate I was made had only a very narrow ectoderm layer, and was overlooked. We may affirm, then, that the eye at all stages of development is a three-layered structure.

Comparison with the Arachnid Simple Eyes.

We are now in a position to make, if possible, a practical application of the preceding. Does a study of the structure and development of the Pycnogonid eye throw any light upon the relationship of the Sea-Spiders to other groups? I think there can be no doubt of this, and that it furnishes a most satisfactory verification of the relationship pointed out in the preceding sections.

It seems absolutely impossible to bring the eyes into the same category with the eyes of the Annelids, which is a decided gain for our position, and it is par excellence an Arthropod eye, and even more, as I hope to show it must be referred to a particular group of Arthropods—the Arachnids. The absence of any very close resemblance to the eyes of Annelids would not be in itself a final argument against Dohrn's position, inasmuch as the eyes may have been much modified during the transition from Annelid to Pycnogonid; but this can scarcely be the case, for the eyes seem to show traces of degeneration in many, if not in all (as shown, perhaps most strikingly, by the large vacuoles, and

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the slight development of lens in some forms), and this could not be accounted for had the group first acquired an eye so different from the Annelids, and afterward, without change of habitat, degenerated. Further, we find a remarkable agreement with the Arachnid eye, and it seems much more probable that this (even alone, but we can consider it now in connection with many other peculiarities, both in structure and development, common to the two groups) has a phylogenetic meaning, and forces one to abandon the theory advanced by Dohrn.

First I must state that the principal papers which I have used in the following comparison are those by Lankaster and Bourne on the Simple Eyes of Limulus, Locy on the Development of the Spider's Eye, and Mark on the same subject, Parker on the Eyes of Scorpions, and Laurie on the Development of the Eye of the Scorpion. Of the more speculative papers of Patten and Watase I shall speak separately.

The adult eyes of the Sea-Spiders show three distinct layers—the corneal hypodermis, the retinal or middle layer, and the posterior or pigment layer. It has been demonstrated beyond doubt that the Arachnid median eyes are composed of three layers, and, further, that these are *more apparent in the embryo than in the adult Arachnid.* Further, the arrangement of the layers is essentially the same, for we find a corneal hypodermis, a middle sensory layer, and a posterior narrow layer.

The middle layer in the embryo Spiders (where the eyes have been studied in most detail) contains at the inner ends of the retinal cells clear (stainable) rod-like bodies, or bacilli, which seem to be identical with the similar bacilli of Pycnogonids. The bacilli in the Pycnogonids are at the inner ends of the cells—in other words, the retina is *inverted*—and in the embryo Spiders we find that similar rods are also at the inner ends of the cells—that is, the retinal elements are here also inverted. A secondary shifting, or change, takes place in the Spider's eyes, so that in the adult the eyes and retina become righted; but how this later change comes about is at present, I believe, unknown.

Lastly, the eyes of the Pycnogonids are formed by a process of invagination of the same nature as the invagination in the Arachnids (Locy, Parker, Laurie, Brooks). This invagination, instead of being an open cup (as in Insects), turns to one side

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and comes to lie under another part of the hypodermis, which forms the outer layer of the eye, and the wall of the invaginated tissue lying outmost forms the middle layer, and the inner the inner layer of the adult eye. The invagination of the cells to form the eye does not take place all at once in the eye of the Sea-Spiders, but is a gradual process, so that a few cells at a time are added to the eye, and this is connected with the presence of a small, free larval form having the beginnings of the eyes. In the Arachnids, on the other hand, the process of invagination is more marked, in that all the cells are invaginated at once. These similarities in development and adult structure, it seems to me, point unmistakably to a community of descent between the Pycnogonids and the Arachnids, and further, they point to the same conclusion which we reached in studying the embryology of the group, that the Sea-Spiders show in their adult structures close resemblances to the embryos of the Arachnids, inasmuch as the inversion of the eye of the Pycnogonid agrees with the inversion of the embryo Spider, and the well-defined three-layered condition of the latter in the embryo is seen fully developed in the eye of the adult Pycnogonids.

Inversion of the Eye.

The early inversion of the eye of the Spiders has been noticed by Locy, and Mark has extended this discovery to the solution of certain theoretical problems. The latter suggested that the process of invagination of the Spider's eye repeats the method by which such an eye arose, that is to say, if we start with a simple cup-shaped invagination and suppose the walls *on one side* to have increased greatly, so that they ultimately filled up the central cavity, rolling over into this cavity, the inversion of the eye was explained. The opposite wall of the cup remaining thin and developing pigment, became the inner layer of the eye, and the general hypodermis of the body formed the secreting cells of the lens or the outer layer. Patten has suggested that the Arachnid eye is but a later stage of development of that seen in the Insect eye. The walls of the optic cup turn in from the *two sides*, the elements at the sides becoming first horizontal, then turning into the cavity of the vesicle, the retinal cells at the bottom of the vesicle forming a posterior (inner) layer to the

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eye. Patten's suggestion was at the time, I think, little more than a suggestion of a possibility, and no structures in the Arachnid eye were given to support it.

Mark, on the other hand, has complicated his hypothesis by certain additions, both of which I believe one would, *à priori*, rather reject, if possible. First, that the one-sided development he supposes to have taken place to have been the result of a change of position in the lens, so that part of the eye was placed to better advantage than the rest, a supposition not founded on any facts, and it seems to me highly improbable. Secondly, the inverted elements of the embryo right themselves (as they are so found in the adult) by a complete change in the parts of each element, so that the rods which are at first secreted at one end of the cell—the inner—subsequently are formed at the opposite end of the element—the outer—and that the nerve fibrils were also transferred from one end of the cell to the other. To be sure we do not yet know how the eye does right itself, yet it seems most improbable it has done so by such an unusual method, and it would be much easier to believe that the elements shift around so that their inner ends become the outer.

I have examined carefully the inverted eye of the Pycnogonids to see if it would throw any light upon the early inversion of the eyes of Arachnids. In the first place, I am inclined to believe that this inversion of the Spider's eye cannot be explained as a purely embryonic phenomenon. From what has been given in the preceding sections, I am ready to believe that this inversion of the embryo Spider's eye is to be explained as a phylogenetic process, and that the adult Pycnogonid's eye represents approximately the condition of the eye of the ancestral Arachnid (or, at any rate, the ancestral Spider).

Let us now see if there is anything in the Pycnogonid eye to determine whether this primitive inversion was caused by a one-sided development of the eye, as Mark has assumed (for Spiders), or whether we have any evidence pointing in other directions. I have been much impressed by the bilateral arrangement of the eye in the Pycnogonids, and it has seemed to me probable that this might give a clue to the solution we are in need of. The corneal hypodermal cells show a marked bilateral structure in the arrangement of the cells on each side of the middle line.

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The middle layer (and this is more to the point) shows a strong bilateral arrangement—first, in respect to the nuclei which lie largely on the two sides, and, secondly, in the arrangement of the retinal elements to the right and left of a median raphe. The third layer has a bilateral arrangement, the cells along the middle being different from those along the sides of the eye, and those at the sides approximating in structure to the retinal elements.

It does not seem to me that it is possible to interpret this bilaterality on Mark's hypothesis, because it furnishes not the slightest reason why an eye so derived should show a two-sided arrangement, nor is it likely to be explained as a secondary arrangement.

On the other hand, if we assume the inversion to have arisen by a turning in of two sides of an optic cup—a process already shown in the Insects' simple eyes—we see clearly why the eye should present so perfectly a bilateral plan. To explain this more in detail, we may start with a simple invagination, forming a thick-walled cup with nerve fibers entering the inner ends of the cells. The early stages of development of Insects' simple eyes show such a condition. Next, the lips of the invagination touch and fuse, forming an almost continuous layer over the eye, giving the corneal hypodermis. The bilateral arrangements of this layer in the Pycnogonids may be the expression of this union from the two sides. At the same time that this occurs, the more horizontally-lying retinal elements in the upper part turn into the cavity of the cup, so that their morphologically outer ends now come to point inwards. The nerve fibers which ran up to the back part of the eye continue to do so, and, running from their point of contact around the outer wall of the cup, supply the elements with fibrils. This is practically the condition of the simple eyes of Insects. Such an eye may be said to be (imperfectly) three-layered at this stage, as we may now count the partially inverted elements as a middle layer. The elements lying at the base of the cup are in a disadvantageous position for vision, being covered by a sensory layer (the inverted fibers) lying between them and the light. So these elements of the bottom of the cup and at the sides degenerate and become a pigment layer at the back of the eye. Now we have reached the type of eye found in the Sea-Spiders.

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The evidence that the eyes of the Sea-Spiders have evolved along some such line as this is found in the structure of the eye as existing to-day. The median raphe of the middle layer indicates the points of contact between the retinal elements from the two sides. These, as they became inverted, came into contact in the middle line. The bilateral arrangement of the nuclei follows almost as a necessity of this two-sided inversion. These would be crowded together when the retinal elements turn in, and few or none along the middle line. Patten shows on the *outer* ends of the retinal elements of Insect eyes a layer of what he calls "secondary nuclei," which, so far as I can judge, are identical with the round stainable bodies found at the *inner* ends of the inverted elements of the Pycnogonid eye. One sees at once that by the inversion of the lateral elements of the cup the bodies must be found in exactly the same position as they are found in the Pycnogonid eye.

If further evidence were needed to prove the two-sided inversion, it would, I think, be amply found in the conditions existing in the pigment layer of the eye. The cells immediately at the back (at the base of the cup) are cylindrical cells, such as would result from a shortening of the retinal elements. If one draws a diagram of the process I have sketched, it becomes evident that as we progress around the sides of the eye the pigment cells must pass insensibly into the retinal cells; and such, indeed, we found previously to be the case. Thus the retinal cells here have not suffered so great a change as those at the back of the eye; but, developing pigment, they furnish a dark background for the retinal cells, but still retain largely their ancestral shape. Finally, the large nerve process running from these pigment cells is explained by their ancestral function as fibrils to the retinal elements.

To sum up, I believe all the layers of the Pycnogonid eye give abundant evidence that the eye has developed by the turning in of two sides of a primitive optic vesicle, and that the simple eyes of Insects furnish all the intermediate stages, both in development and adult structure, between a simple cup-like invagination and the three-layered condition of the Pycnogonid eye.[1]

[1] This and several other facts (as multipolar delamination, etc.) point perhaps to a genetic relationship of Insects to Arachnids, but I cannot go into these comparisons at present.

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I am inclined to push this a step further and attempt to show that the median Arachnid eyes have developed along a similar path, and this must be a necessary corollary to the position to which I have assigned the Pycnogonids, and believe, as I have before said, that the inversion of the Spider's eye is its Pycnogonid stage of evolution and the righting of it a secondary and later change. I also believe there is no evidence that in the evolution of the Spider's eye a change of position in the lens has taken place.

General Consideration of the Simple Eyes.

Before leaving the subject there are two points to which I must refer—the meaning of the invagination of Arachnid (and Pycnogonid) eye, and the explanation of the grouping together of the retinal cells into bundles or retinulæ.

It will be at once seen, with respect to the first point, that I have placed more faith in the evidence furnished by the adult eye of the Pycnogonids and Spiders than in that given by the ontogeny of those eyes, while Mark has utilized the ontogeny as furnishing a basis for his considerations.

It seems to me that the development of the eye, as we find it in the Pycnogonids, is entirely an ontogenetic process, *i. e.*, it represents here an abbreviated condition. Instead of recapitulating all the early stages, forming first a cup, then turning in the walls, it performs the whole process at one time and thus complicates the matter. In the Pycnogonids the presence of the eyes in all the larval stages, presumably functional in each, must have changed the original process to a very great extent.[1] Yet through all these stages we have been able to recognize the three-layered condition of the eyes. We must believe, from what has been said of the growth of the eye, that in the Pycnogonids the invagination (of the Arachnid type) has been retarded, so that, one end of the invagination being formed, the inturned and inverted cells have functioned as larval eyes, and as the animal increased in size the invagination kept pace, adding more and more cells to the layers of the eye, so that all of the stages had presumably functional eyes, and at the same time the larva retained the original type of Arachnid invagination.

[1] We do not know whether the ancestral Arachnids had free-living larvæ or not.

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It may not be out of place to say a few words about the formation of bundles of elements in the eyes of many Arachnids, as the preceding may have some bearing on the question. In several of the Arachnid median eyes the retinal elements group themselves into bundles of a varying number of cells—the retinulæ. Watase has advanced the idea that these bundles represent each an invagination—a pit—of ectoderm, and represent the process of formation of the eye elements—ommatidia. He homologizes the retinulæ of the simple eyes with the retinulæ of the compound eye; in the latter he also believes they represent ancestral invaginations. I do not care to carry my comparisons into the compound eye, but shall speak only of the simple eyes, in which, I think, there is not sufficient evidence that these bundles each represent an invagination.

It seems most probable that these groups of cells forming retinulæ represent secondary changes in the eye. If the Arachnid eyes have, as I believe, come along some such lines as have been just sketched, then it is evident that the eyes have several times undergone profound changes of which the median Arachnid eye to-day is the outcome. The Insects' eyes show no such complicated groupings of cells as do the Arachnid eyes. The Pycnogonid eyes show no trace of such bundles of cells. And by no means do all the Arachnid eyes show this, as I have satisfied myself in the Spiders. So, unless one had preconceived ideas to apply to the simple eyes, I see no reason for supposing such a combination of cells to represent an invagination. On such a theory the clear central part of the bundles—the center being formed by the union of the inner parts of the cells forming the group—represents the original chitin secretion of the hypodermal cells. I can find no evidence to support this assumption, although I have examined carefully, both by sections and macerations, the retinula-bundles of several Arachnids. The clear central part—the rhabdome—*is not a secretion from a cell, but only a clear protoplasmic* (?) *portion of it*, and is included in the general wall of the cell. Nor is there any evidence that it is of a chitinous nature, for to micro-chemical reagents it acts entirely differently. Further, an apparently similar substance is formed *at the sides* of part of the retinal elements of both Insects and Pycnogonids, and although in the former the cells are in the proper position,

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this clear stainable portion is *at the sides* and not *at the ends*, for a thick protoplasm lies between the rods. Finally, we find similar transparent and perhaps more solid parts of the retinal elements of other eyes belonging to quite different groups, as in the Ascidians and in the Mollusks (see Pecten), and certainly in the latter group no one will imagine them chitinous or any homologue of chitin.

LIST OF WORKS REFERRED TO.

1. Balfour. Notes on the Development of the Araneina. Quart. Journ. Micr. Sci., vol. XX, 1880.

2. Balfour. Elements of Embryology. 1880.

3. Claus. Crustaceen-Systems. Wien, 1876.

4. Dohrn, Ant. Neue Untersuchungen über Pycnogoniden. Mitth. a. d. Zool. Stat. Neapel, Band I, 1879.

5. Dohrn, Ant. Die Pantopoden des Golfes von Neapel. Fauna und Flora des Golfes von Neapel. Band III, 1881.

6. Hatcheck. Beiträge zur Entwicklungsgeschichte der Lepidopteren. Jena, Zeit. Naturwiss., No. 11, 1877.

7. Heider, K. Ueber die Anlage der Keimblätter von Hydrophilus piceus. Abh. d. K. Preuss. Akad. d. Wiss. Berlin, 1885.

8. Müller, Fritz. Für Darwin. Leipzig, 1864.

9. Metschnikoff, E. Entwicklungsgeschichte des Chelifer. Zeit. f. wiss. Zool., XXI, 1871.

10. Schimkewitsch. Zur Entwicklungsgeschichte der Araneen. Zool. Anz., VII, 1884.

11. Schimkewitsch. Étude sur le développement des Araignées. Archiv de Biologie, 6, 1887.

12. Sedgwick, A. A Monograph of the Development of Peripatus Capensis. Stud. Morph. Lab., Camb., IV, Pt. I, 1888.

13. Semper. Ueber Pycnogonida und ihre in Hydroiden schmarotzenden Larven-formen. Arb. a. d. Zool. Zoot. Inst. Würzburg, 1, 1874.

14. Wheeler. The Embryology of Blatta Germanica and Doryphora Decemlineata. Jour. Morph., III, 1889.

15. Wilson, E. B. The Pycnogonida of New England and Adjacent Waters. U. S. Commissioner's Report, Pt. VI, 1878.

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REFERENCE LETTERS FOR PLATES.

B. Brain.
D. Mid-gut, mesenteron.
D^{I}, D^{II}, D^{III}, D^{IV}, etc. Diverticula of mid-gut.
E. Ectoblast.
N. Entoblast.
M. Mesoblast.
P. Periphery of blastoderm.
S. Schizocoels.
V^{1-7}. Ventral ganglia 1—7.
H. Heart.
L. Legs.
O. Opening ventral organs.
C. Central cavity of segmenting egg.

DESCRIPTION OF PLATES.

PLATE I.

FIGURE 1. Section of segmented egg and membranes of Pallene empusa.

FIG. 2. Section of another egg of Pallene at same stage to show central cavity *C*.

FIG. 3. Section through upper pole of egg of Pallene. First formation of blastoderm.

FIG. 4. Section through upper pole of egg of Pallene. Later stage than last. Inner cells—entoblast—are present.

FIG. 5. Section through egg of Pallene. Later stage than last. Cap of cells—blastoderm—covers more of egg than in last.

FIG. 6. Section through periphery of blastoderm, at *P* of Fig. 5.

FIG. 7. Section in same region as last.

FIG. 8. Section of segmenting egg of Tanystylum orbiculare, to show pyramidal shape of cells.

FIG. 9. Section of egg of Tanystylum, to show process of multipolar delamination.

FIG. 10. Same, with delaminated entoblast.

FIG. 11. Section of segmenting egg of Phoxichilidium maxillare.

FIG. 12. Same, to show degeneration of endoderm.

FIG. 13. Cross-section of the Pantopod-larva of Tanystylum orbiculare.

FIG. 14. Circumœsophageal ring of same.

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FIG. 15. Eyes of same.

FIG. 16. Ventral organs of same. Early stage of third pair of ganglia.

PLATE II.—SECTIONS OF PALLENE EMPUSA.

FIG. 17. Longitudinal section through stomodæum of young embryo. First origin of mesoblast at *M*.

FIG. 18. Cross-section through stomodæum of older stage. See line 18, Fig. I, Plate IV.

FIG. 19. Section anterior to last. See line 19, Fig. I, Plate IV.

FIG. 20. Section anterior to Fig. 19. See line 20, Fig. I, Plate IV.

FIG. 21. Cross-section of ventral organs. See line 21, Fig. I, Plate IV.

FIG. 22. Cross-section of ventral organs at stage III.

FIG. 23. Cross-section of ventral organs at stage V.

FIG. 24. Cross-section of ventral organs at stage VI.

FIG. 25. Cross-section of embryo at stage III.

FIG. 26. Cross-section through brain and second pair of ventral ganglia of stage III.

FIG. 27. Cross-section of embryo in the plane of a pair of legs, about stage VI.

FIG. 28. Cross-section of embryo in the plane between a pair of walking legs, about stage VI.

FIG. 29. Cross-section through the rudimentary abdomen and the body of an embryo, stages VII–VIII.

PLATE III.

A–J, Pallene empusa. *a–c*, Phoxichilidium maxillare. *d–f*, Tanystylum orbiculare.

FIG. A. Segmenting egg, side view, 2-celled stage.

FIG. B. Segmenting egg, seen from above, 4-celled stage.

FIG. C. Segmenting egg, seen from above, 8-celled stage.

FIG. D. Segmenting egg, seen from below, 8-celled stage.

FIG. E. Segmenting egg, side view, 16-celled stage.

FIG. F. Segmenting egg, surface views of micromeres, 16-celled stage.

FIG. G. Segmenting egg, side view, 16 macromeres, 8 micromeres.

FIG. H. Segmenting egg, side view, 24 macromeres, 16 micromeres.

FIG. J. Segmented egg, lower pole, base of pyramidal cells shows at surface.

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FIG. a. Phoxichilidium, 2-celled stage.
FIG. b. Phoxichilidium, 4-celled stage.
FIG. c. Phoxichilidium, 8-celled stage.
FIG. d. Tanystylum orbiculare, 2-celled stage.
FIG. e. Tanystylum orbiculare, 4-celled stage.
FIG. f. Tanystylum orbiculare, 32 (?)-celled stage.

PLATE IV.

I–VIII, Pallene. IX, Tanystylum.

FIG. I. Surface view of young embryo, to show anterior region.

FIG. II. Surface view of ventral region of same.

FIG. III. Ventral view of embryo with four pairs ventral ganglia, first and second separate.

FIG. IV. Dorsal view of embryo about same age (a little older perhaps) as last.

FIG. V. Ventral view of embryo. First and second ventral ganglia have fused.

FIG. VI. Side view of embryo, showing rudimentary abdomen behind as a continuation of the body and in same plane as the body.

FIG. VII. Ventral view of embryo at the age when it leaves the parent. Fourth pair of walking legs seen behind; third pair of appendages beginning just in front of first pair of walking legs.

FIG. VIII. Dorsal view of last, to show brain and four eye spots. In both VII and VIII only the proximal ends of the legs are shown.

FIG. IX. The Pantopod-larva (Protonymphon of Hoek) of Tanystylum orbiculare.

PLATE V.—METAMORPHOSIS OF TANYSTYLUM. Zeiss 4 A ½.

FIG. X, stage 2. Young larva after leaving parent and after first (?) moult. Ventral view.

FIG. XI, stage 2. Dorsal view of last.

FIG. XII, stage 3. Ventral view.

FIG. XIII, stage 4. Ventral view, showing three pairs of ventral ganglia, the first really composed of two.

FIG. XIV, stage 4. Dorsal view of last. One pair of eyes—the anterior.

FIG. XV, stage 5. Ventral view with first pair of walking legs, each with six segments.

FIG. XVI, stage 6. Ventral view with two pairs walking legs, the first with eight segments, the second with six.

FIG. XVII, stage 7. Ventral view with three pairs walking legs,

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the first with eight segments, the second with eight, the third with six.

FIG. XVIII, stage 8. Ventral view with four pairs walking legs, the first with eight segments, the second with eight, the third with eight, the fourth with six.

PLATE VI.—LARVÆ AND ADULT OF TANYSTYLUM. LARVA OF PHOXICHILIDIUM. LARVA OF PALLENE.

FIG. XIX, stage 9. Tanystylum. Ventral view. Showing renewed growth of third pair of appendages. Zeiss 4 A ½.

FIG. XX, stage 9. Dorsal view of last. Showing rudiments of first appendages. Zeiss 4 A ½.

FIG. XXI, stage 10. Tanystylum. Ventral view. Showing segmentation of third appendages, and fusion of first, second and third ventral ganglia. Zeiss 4 A ½.

FIG. XXII. Adult Tanystylum. Dorsal view. Proportionately less magnified.

FIG. XXIII. Ovigerous leg and eggs of Tanystylum. Zeiss 2 A ½.

FIG. XXIV. Adult Phoxichilidium. Side view of male with eggs. × 30.

FIG. XXV. Pantopod-larva of Phoxichilidium.

FIG. XXVI. Larval Pallene. Ventral view. Showing the undeveloped third pair of appendages.

PLATE VII.—EYES OF PHOXICHILIDIUM, 30–36. PALLENE, 37. TANYSTYLUM, 38–39. Zeiss 4 D ⅔, except Fig. 31.

FIG. 30. Eye of Phoxichilidium, viewed on the inner side, from a macerated preparation. Showing distribution of nerve to eye and bilateral arrangement of elements along the raphe.

FIG. 31. Section across cupola, containing the eyes which are cut into "cross sections" (horizontal with reference to body).

FIG. 32. Cross-section of eye as in 31, but more magnified.

FIG. 33. Longitudinal section of eye, parallel to raphe.

FIG. 34. Section in plane at right angles to other two. Showing bacillar ends of the elements of the retina, forming by their union the raphe.

FIG. 35. Section in same plane, but further out than last.

FIG. 36. Section in same plane, but nearer the center of the eye.

FIG. 37. Longitudinal section of eye of Pallene.

FIG. 38. Longitudinal section of eye of Tanystylum.

FIG. 39. Inner view of cells forming raphe of Tanystylum.

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PLATE VIII.—MACERATIONS OF EYE OF PHOXICHILIDIUM. DEVELOPMENT OF EYE OF TANYSTYLUM AND SECTION OF GANGLIA OF SAME, ETC.

FIG. 40. Retinal elements of eye of Phoxichilidium obtained by maceration in Haller's fluid.

FIG. 41. Others of same.

FIG. 42. Collection of three retinal elements, from maceration.

FIG. 43. End of cell showing bacilli, from a maceration in sulphuric acid and sea-water.

FIG. 44. Retinal cells of outer surface of middle layer of Pallene.

FIG. 45. Pigment cells of inner layer of Phoxichilidium.

FIG. 46. Eye of Tanystylum-larva. Longitudinal section of anterior and only pair. Stage 4. Zeiss 4 F ½.

FIG. 47. Same of older larva. From posterior pair. Stage 5.

FIG. 48. Same of one posterior eye of older larva. Stage 6.

FIG. 49. Same of one posterior eye of older larva. Stage 7.

FIG. 50. Section in same series as last.

FIG. 51. Same of older larva. Stage 8. Zeiss 4 F ½.

FIG. 52. Cross-section through posterior part of body of larval Tanystylum, showing beginning of a pair of ganglia, etc. Zeiss 4 D ½.

FIG. 53. Pair of ganglia with ventral organs disappearing.

FIG. 54. Pair of ganglia with cavities of ventral organs. Zeiss 4 D ½.

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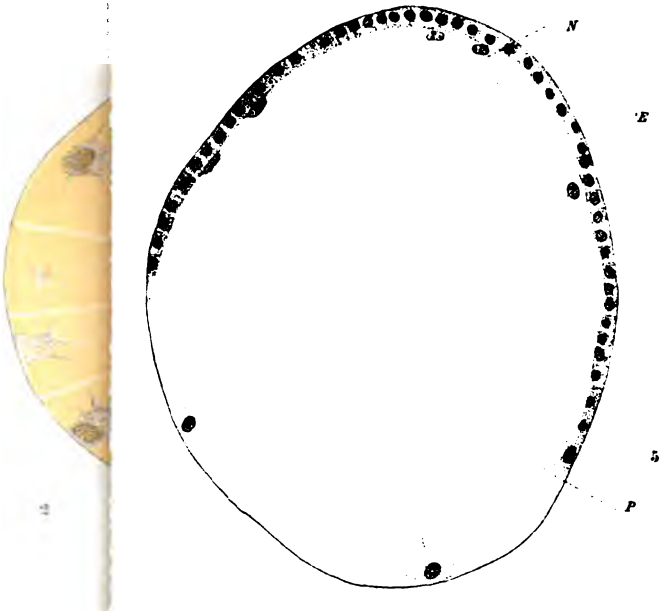

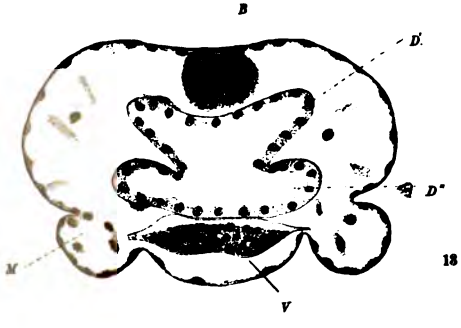

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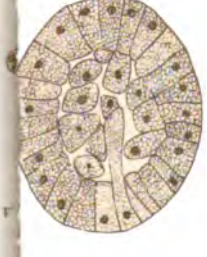

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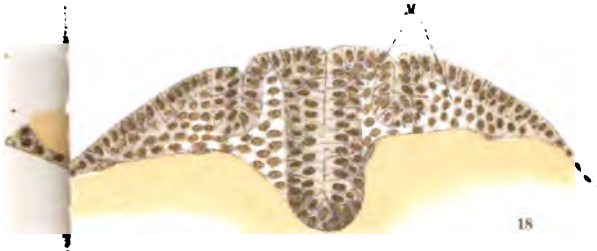

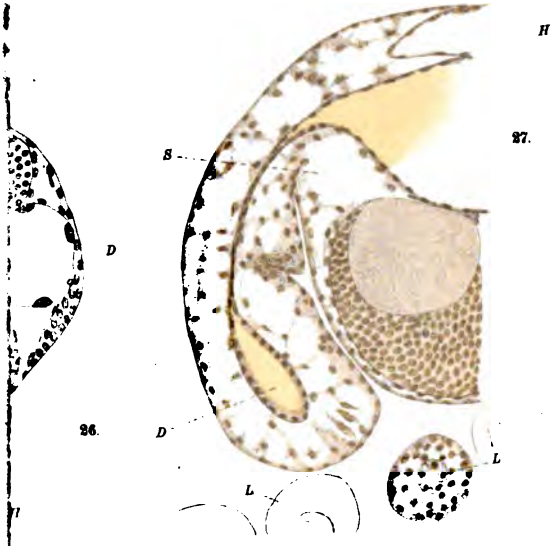

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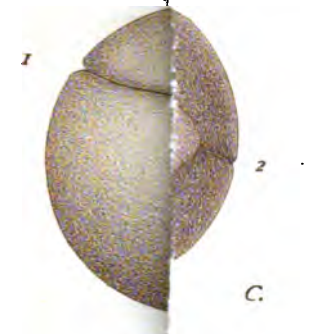

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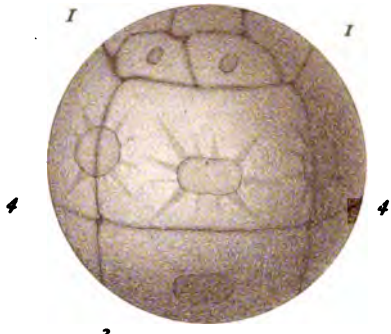

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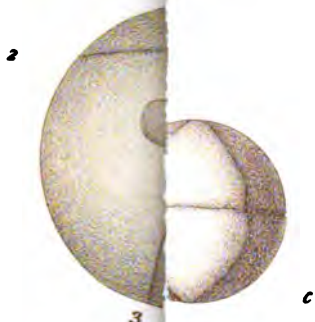

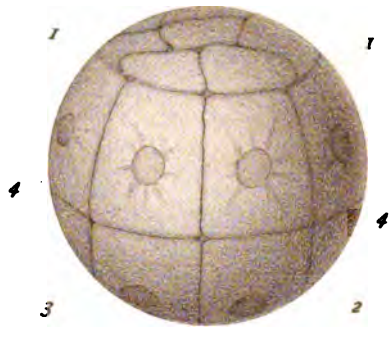

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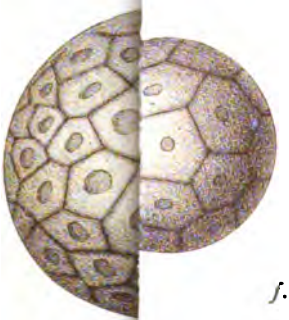

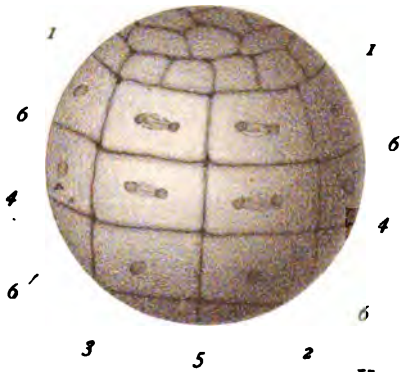

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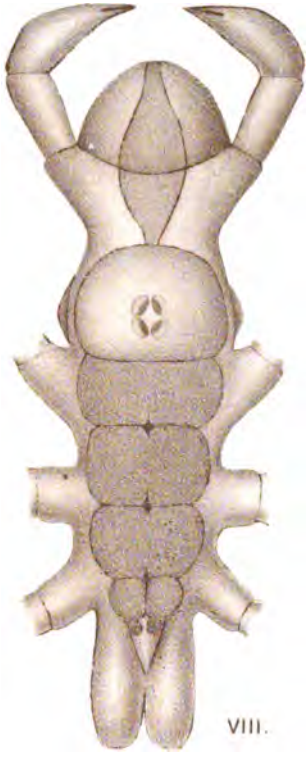

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XIII.

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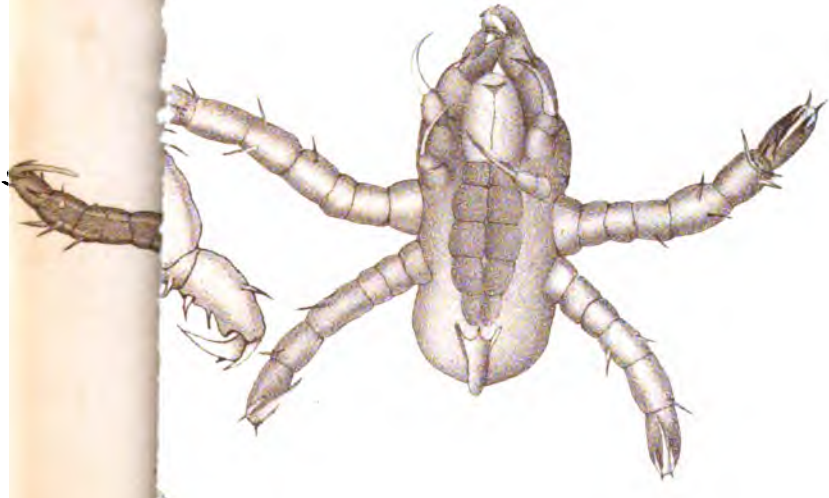
XI.



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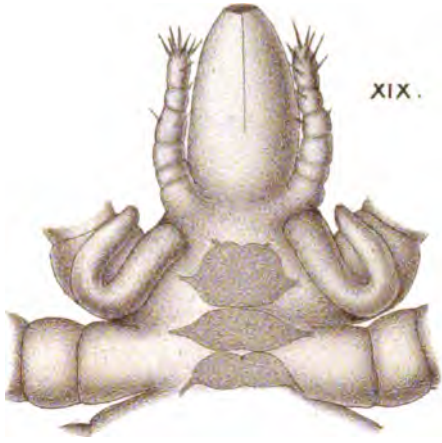

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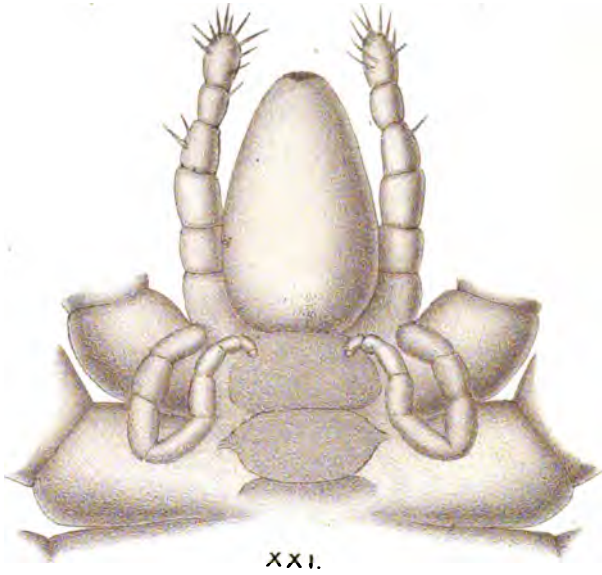

XXI.

XXIII.

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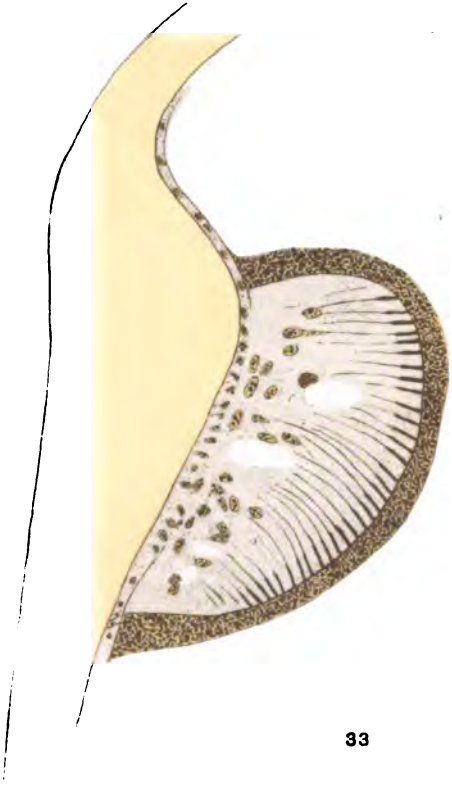

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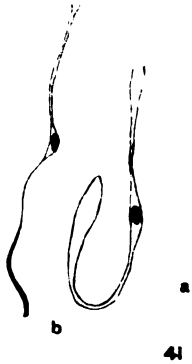

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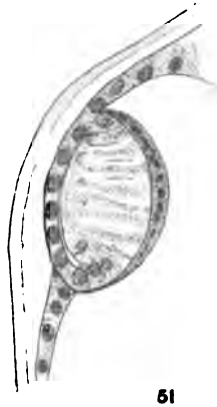

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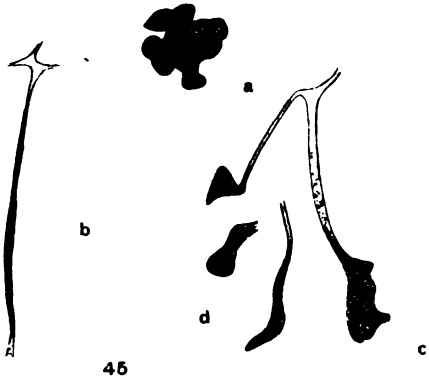

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THE EFFECTS OF HEMORRHAGE AND OF FASTING ON THE PROTEIDS OF THE BLOOD OF CATS.

By GEO. P. DREYER, B. A., Ph. D., Associate in Physiology, Johns Hopkins University.

I.—Introductory.

Alex. Schmidt was the first to recognize the presence of three distinct proteids in the blood, and this discovery naturally led to the notion that each had its own special rôle in the metabolisms of the living body. It therefore became important to devise means for separating them and estimating the amount of each.

Hammarsten partially attained this end when he discovered that magnesium sulphate, added to saturation, completely precipitated serum globulin from serum.

To estimate the fibrinogen he found more difficult; he was compelled to rely on the determination of the amount of fibrin any specimen of blood gave, and from that to calculate the amount of fibrinogen originally present. This method was subsequently adopted by other workers as the best available, although its imperfections were recognized. Hammarsten[1] himself proved that all the fibrinogen is not precipitated as fibrin during coagulation, and, further, that the ratio between a quantity of fibrinogen and the fibrin resulting from it is not constant, but depends on a variety of conditions, such as the amount of alkali or salts that may be present.

In 1877 Fredericq[2] proposed a new way of determining the fibrinogen of the blood, a method suggested to him by Kühne's "Fractional Heat-Coagulation" method as used by that author in his experiments on muscle plasma. The applicability of the method to the analysis of the blood depends on the wide separation of the heat-coagulation temperature of fibrinogen, 56° C., from that of serum globulin and of serum albumin, which lies between 70° C. and 80° C. Fredericq found that it was only necessary to prevent the blood from clotting by any of the well-known methods

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and to heat it to 56° C. for a few minutes in order to bring about a complete precipitation of the fibrinogen. He got fairly good results by the method; for instance, in the blood of a horse which gave .375 per cent of fibrin he found .4299 per cent of fibrinogen by heating to 56° C. That is to say, his heating method gave a considerably heavier precipitate of fibrinogen, and, therefore, one more nearly theoretically correct than that produced normally in coagulation, which is known to be incomplete. In three equal portions of a salted plasma which were heated to 65°, 61.5° and 56° respectively, he obtained .2878 gram, .295 and .2668 gram of fibrinogen. The limit of error certainly is smaller here than in the older method of calculating the fibrinogen from the fibrin.

A year or two later Hammarsten,[1] after thoroughly investigating the properties of fibrinogen, claimed that, just as in ordinary coagulation only a part of the fibrinogen is converted into the insoluble fibrin, while another portion remains in solution as a globulin; so also when heated to 56° C. is the fibrinogen split up into a soluble and an insoluble product.

If this observation be correct, the new method is no improvement on the old. But subsequent work has not confirmed the assertion. Howell[8] found that the fibrinogen obtained from blood of the slider-terrapin is completely precipitated between 56° and 60° C.

It might be supposed that this difference in the results was in some way connected with the fact that Howell obtained his fibrinogen from a cold-blooded animal, while Hammarsten worked with mammals. But Howell showed that the same thing was true for fibrinogen prepared from hydrocele fluid.

I have tested this matter for cat's blood, and find that here, too, the fibrinogen all comes down on heating to between 56° and 60° C. Sometimes the xantho-proteic reaction indicated a trace of albumin in the filtrate, but even this failed to appear when a single drop of .5 per cent solution of acetic acid was added to the neutral solution of pure fibrinogen before heating it.

On the basis of the foregoing considerations, the method which I adopted for analyzing the blood was briefly the following: The blood is taken from the carotid artery into cylinders surrounded

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with ice and containing a saturated solution of $MgSO_4$. Approximately, one part of $MgSO_4$ was allowed for every 10 parts of blood. This is sufficient to prevent clotting, while the corpuscles subside more readily than when larger quantities are used. By means of the centrifuge the clear salted plasma may be obtained in about two hours, and the analysis started on the same day. 10 ccm. of the plasma are neutralized by the addition of one per cent acetic acid, and, finally, by an extra drop, a faint acid reaction is made to appear. On now heating to 60° C. for five minutes the fibrinogen comes down. It is collected on a weighed filter, washed free from all other proteids, salts and fats, and finally heated to a constant weight at 110°. On correcting for the $MgSO_4$ present the result gives the fibrinogen contained in 10 ccm. of blood.

Five ccm. of the filtrate from the first precipitate are then saturated with powdered $MgSO_4$ at a temperature of 35° C. to bring down the serum globulin. This determination must be corrected for the volume of added acetic acid and of $MgSO_4$ solution. To facilitate this, the acetic acid used in neutralizing the plasma was dropped from a graduated pipette. Finally all the proteids are precipitated from 5 ccm. of the salted plasma by saturation with $(NH_4)_2SO_4$. The filtrates from these precipitates were tested in every case, but no trace of albumin was ever found in them. The serum albumin can thus be determined by subtracting the serum globulin and fibrinogen from the total proteids.

Two parallel analyses were always made in order to control the results. Unless the agreement was quite close, the analysis was rejected. On this account only the averages are given in the tables below. I ought to add that the agreement was always very close except when some recognized accident accounted for a more marked difference.

II.—Anæmia.

To ascertain the changes produced in the blood by hemorrhage the cats were bled twice at an interval of 24 hours. The extent of the first bleeding varied from 55 ccm. to 81 ccm.; at the second bleeding they were bled to death. Both specimens of blood were then analyzed in the manner already described, giving the following results:

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TABLE I.

Proteids in 100 cub. cent. of blood.

Cat.		Normal.	After Bleeding.	Extent of 1st Bleed.	Increase of Fibrinogen.
I.	Fibrinogen,	.357	.396	55 ccm.	.11
	Total proteids,	5.014	5.907		
II.	Fibrinogen,	.232	.374	81 ccm.	.61
	Total proteids,	5.995	5.555		
III.	Fibrinogen,	.153	.340	58 ccm.	1.22
IV.	Fibrinogen,	.215	.365	63 ccm.	.70
	S. glob.,	3.436	3.951		
	S. alb.,	2.404	1.536		
	Total proteids,	6.055	5.852		
V.	Fibrinogen,	.222	.297	68 ccm.	.43
	S. glob.,	4.614	2.885		
	S. alb.,	2.387	3.718		
	Total proteids,	7.223	6.901		
VI.	Fibrinogen,	.233	.382	67.5 ccm.	.64
	S. glob.,	2.880	1.684		
	S. alb.,	2.885	3.821		
	Total proteids,	5.998	5.888		
VII.	Fibrinogen,	.381	.417	81 ccm.	.10
	S. glob.,	3.431	3.447		
	S. alb.,	2.894	2.619		
	Total proteids,	6.707	6.483		
	Average.				
A.	Fibrinogen,	.256	.367		.43
	S. glob.,	3.590	2.992		
	S. alb.,	2.643	2.924		
	Total proteids,	6.165	6.098		

Section A does not represent an actual experiment, but was obtained by averaging the corresponding items of all seven cats used; giving thus average fibrinogen from all the fibrinogens, etc.

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Since the first three experiments are incomplete and give no figures for the serum albumin and serum globulin, the total proteids in the average cat are not equal to the sum of fibrinogen, serum albumin and serum globulin as in the other cases. In cats I, II and III the analyses are incomplete owing to accidental loss of the remaining precipitates.

Even a superficial inspection of the table suffices to show that considerable variation occurs in all the constituents of the plasma in normal cats.

Fibrinogen on the whole was pretty regular, falling for the most part between .20 and .23 gram in the hundred cubic centimeters of blood. But in Nos. I and VII it went up to .357 and .381 gram.

The serum albumin varied least, its minimum being 2.387, its maximum 2.894 grams in the hundred cubic centimeters.

The serum globulin is much less regular.

The history of the cats prior to their use was not known, but it is probable that they were in different states of health and nutrition, which would account for the above differences.

Equally great differences are on record for other animals. Thus Howell[3] found the serum glolulin of terrapins to vary from 3.181 to 4.777 per cent, and the serum albumin from 0.42 to 1.004 per cent of the blood. In the dog, according to Mayer,[4] the fibrin obtained from 100 ccm. of blood ranges from $\left\{\begin{matrix}0.066\\0.081\end{matrix}\right\}$ to $\left\{\begin{matrix}0.349\\0.351\end{matrix}\right\}$ gram, and he expressly adds that the dogs were normal as far as he could tell. These variations are larger than those observed by me on cats.

Hammarsten determined the total amount of proteids, and also the relative proportions of serum globulin and serum albumin, in 100 parts of the serum obtained from the blood of the horse, ox, man, and rabbit.

He gives the following table of his results:

	Total Proteids.	S. Glob.	S. Alb.	Ratio Serum Globulin to Serum Albumin.
Horse,	7.257	4.565	2.677	1 : .59
Ox,	7.499	4.169	3.329	1 : .84
Man,	7.619	3.103	4.516	1 : 1.51
Rabbit,	6.225	1.788	4.436	1 : 2.5

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The cat, according to my analysis, gives:

Total Proteids.	S. Glob.	S. Alb.	Ratio Serum Globulin to Serum Albumin.
6.165	3.590	2.643	1:0.74

The cat is therefore nearest to the rabbit as far as the total proteids are concerned, while the ratio of s. glob. to s. alb. lies between that of the horse and ox. In reality the serum globulin of the cat is given a little too low in comparing it with Hammarsten's table, because he analyzed serum in which the serum globulin appears with the increase which it receives during coagulation.

The Total Proteids.—In all except cat I the percentage of these is found to be diminished at the second bleeding.

Bizzozero[5] found that the sudden diminution of the total proteids, produced by blood-letting, is gradually followed, not only by a return to the normal, but even by a decided increase.

A similar increase above the normal was recently found to occur in dogs by Favilli.[6] He always allowed an interval of several days between successive bleedings, and this fact, I think, accounts for the difference in our results. As the interval in my cats was never more than 24 hours, the second bleeding occurred before the rise in the proteids was able to reach the normal condition. In cat I, however, this was not the case, because the first loss of blood was less severe than in the others and consequently the proteids had had time enough to return to and pass beyond the normal amount.

The serum globulin and serum albumin do not seem to follow any fixed law. Sometimes one, sometimes the other is increased by the bleeding.

What it is that determines that in one case serum globulin and in another serum albumin should be more abundantly formed I do not know. Both of them can be derived from the morphological elements of the body, which, after severe bleeding, seem to dissolve and by a process of reversed assimilation pass back into the blood. The cats received no nourishment in the interval between the two bleedings, so that the new supply of proteid substances must have been derived from the organized material of the body.

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The same thing has been found true for dogs, in which, according to Salvioli, both serum albumin and serum globulin tended to return to the normal after repeated loss of blood during prolonged starvation. It seems, therefore, that Favilli[6] is in error when he asserts that, after loss of blood, the serum albumin is restored by assimilation of food material from the alimentary canal, while only serum globulin is derived from the tissues by absorption through the capillaries and lymph channels.

Fibrinogen.—Hemorrhage increased the percentage of fibrinogen in every case, the increase varying from 10 to 122 per cent of its original amount.

In one cat I determined the fibrin instead of the fibrinogen and found a similar increase. Thus cat VIII gave on first bleeding 0.268 gram fibrin in 100 ccm. of blood, and 24 hours later 0.324 gram, an increase of 21 per cent.

A similar observation has been made on dogs by Mayer. He bled the dog at intervals of two and eight days and found on analysis .216, .412, .550 gram fibrin per 100 ccm. of blood.

How is this increase of fibrinogen to be explained? It is well known that after venesection, by which the number of red corpuscles is much reduced, they are replenished with such rapidity that the blood may again contain the normal number per cubic millimeter in the course of a few days. It is for this reason that those who desire to examine the development of red corpuscles generally resort to the device of bleeding the animal, thereby exaggerating the normal process.

It is now generally agreed that these red corpuscles are formed from special hæmatoblastic cells. Differences of opinion exist as to the details of the process; according to Malassez, each hæmatoblast gives rise to a number of corpuscles which are split off as buds.

According to another view, however, the nucleus is bodily extruded from the hæmatoblast, the rest of the cell becoming a single red corpuscle. Howell,[14] working in this laboratory, confirmed the latter view, and the desire to discover what became of the nuclei of the hæmatoblasts was the chief purpose of my analyses. It is known that the nuclei quickly go into solution in the lymph or blood, and the most natural supposition is that they give rise

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to one or more of the albumins which are present there. If this be true, then any process that intensifies the hæmatopoietic function of the body should at the same time produce a corresponding increase of that or those proteids of the plasma which are derived from the solution of the nuclei of hæmatoblasts. Since we find that bleeding, which sets up a rapid regeneration of red blood corpuscles, in every case produces also a marked increase of fibrinogen, there is reason to believe that this fibrinogen comes from the dissolved nuclei of hæmatoblasts.

Fibrinogen is normally present in so small a quantity, that we can hardly account for its occurrence in the blood on the score of its nutritive value. But on the assumption made above as to its origin, not only can its constant presence in such small quantity be explained, but also its rapid increase after hemorrhage. For the red blood corpuscles, like every other morphological element of the body, are all the time dying; various pigments of the body are sufficient evidence of this. To make good this loss a corresponding development must be going on. By our hypothesis this implies a constant supply of fibrinogen for the blood. When by venesection the manufacture of corpuscles is augumented, a corresponding increase of fibrinogen is a necessary consequence.

In 1875 Alex. Schmidt[7] described some observations first made by his pupil, Semmer,[7] on the blood of amphibia and birds, and afterwards repeated by himself. Namely, they noticed that the defibrinated blood of these animals, when diluted with four to six times its volume of water, or, better still, of a dilute ferment solution, will give a second clot. Such a second clot was not produced by the serum when treated in the same way; indeed, the second clot obtained from the corpuscles was increased when care was taken to remove the serum before the water was added. The fibrinogen necessary for this second clot must have been obtained from the corpuscles.

When Schmidt tried the same experiment on mammalian blood he never succeeded in getting a second clot. Now, as far as we know, the chief difference between the red corpuscles of mammalia and those of birds and amphibia is the presence of a nucleus in the latter. The nuclei of the avian and amphibian corpuscles can account for the second clot, which implies that they first gave rise

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to fibrinogen. If the transformation of the nuclei is possible in this case, there is no reason to suppose that it would be more difficult for the extruded nuclei of the hæmatoblasts of mammals, when they are thrown into the lymph or blood stream.

The above theory of the origin of fibrinogen explains an observation made by Franz Boll.[8] Boll found that the blood of chick embryos, when carefully drawn so as to avoid all contamination, does not clot until the embryo reaches a certain age; that is to say, the twelfth day of incubation. Blood obtained from embryos a little older showed gradually increasing amounts of fibrin until, at about the sixteenth day, a regular clot is formed.

On testing the blood of those young embryos which failed to coagulate, with pericardial fluid and with cruor of frog's blood, he found that it lacked fibrinogen. The pericardial fluid at once set up coagulation, the cruor left it unchanged.

We have already referred to the fact that destruction and regeneration of the red blood corpuscles go on simultaneously. Still we must suppose that each individual corpuscle lives for some time, during which it contributes its share to the maintenance of the organism. The length of life of the corpuscles must no doubt depend on many circumstances; no determinations of its approximate length have come to my notice. However, I do not think it will do violence to any established facts to suppose that it may equal about 10 or 12 days in embryo chicks. If this were so, it would not be difficult to explain Boll's observation. The red corpuscles of the chick being nucleated, no fibrinogen is produced during their development, and the blood cannot clot until such time as some of them begin to break down and liberate their nuclei.

My theory for the origin of fibrinogen is thus seen to be in harmony with many facts and to give a satisfactory explanation of them. It would be desirable to give it a firm support on a chemical basis, but in the present state of our knowledge of the chemistry of the proteids it can hardly be hoped to trace those relationships between the constituents of the nucleus and the fibrinogen of the blood which would account for the ready transition of the former into the latter.

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III.—Fasting.

It has been shown that considerable variations occur in the relative quantities of the several proteid constituents of blood plasma not only among different species, but also among the individuals of the same species.

When Tiegel[9] observed that starved snakes lost all their serum albumin, while feeding restored it, it seemed that one very important factor in producing changes in the blood had been discovered.

Moreover, N. Popoff[10] and J. Brinck[10] recently found that a peptone solution, which is known to be incapable of nourishing the isolated frog's heart, gains the power to do so after a sojourn in the intestines. That is to say, the intestines in some way convert peptone into serum albumin. For the peptone solution had not only gained the power to act as food for the frog's heart, which in itself is considered a characteristic reaction of serum albumin, but it had acquired all the other properties of that substance.

Putting together these facts, it would seem that the proteid constituents of the food are absorbed for the most part as serum albumin, and that this body plays the chief part in nutrition.

But as Howell[3] pointed out, Tiegel's observation ought to be received with some caution, since he precipitated his serum globulin by Panum's method, which is known to give but an imperfect separation, so that boiling should have given him a precipitate even if no serum albumin had been present.

Burckhardt[11] thought he could confirm the results of Tiegel on dogs. But in his case also the method lacked accuracy; he depended on dialysis for the separation.

On the other hand Howell,[3] who worked with terrapins and used Hammarsten's method of precipitating serum globulin, concludes that "feeding increases but slightly, if at all, the quantity of serum albumin present."

Salvioli[12] goes even farther; he finds on comparing the blood of two fasting dogs with that of two others in a state of active digestion, that the serum globulin is diminished in fasting, and not the serum albumin.

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Having determined the composition of the normal cat's blood in connection with the first part of my work, it seemed to me that this opportunity ought not to be allowed to pass by without determining the effect of starvation on the albumins of cat's blood. For we can only hope to straighten out the contradictory results obtained by different workers by extending the observations to a large number of animals.

The cats on which I worked received no food for two weeks, but were not deprived of water. Two of them were weighed before and after the fast; one of them had lost 520 grams, the other 730 grams of the total body weight. Post-mortem examination showed that all of them had retained a considerable quantity of fat.

The blood of the cats was drawn and analyzed in the same way as before, and the results of the analysis brought together in Table II must be compared with those of the first column of Table I. Here, too, the figures given in the table, indicating so many grams per 100 ccm. of blood plasma, are the averages of two parallel analyses in every case.

TABLE II.

Cat.	Period of Starving.	Fibrinogen.	S. Glob.	S. Alb.	Total Proteids.	Loss in Weight.
9	14 d's	.1928	2.256	2.848	5.296	Not Observed.
10	"	.257	.837	3.858	4.952	"
11	"	.314	2.002	2.270	4.585	"
12	"	.271	2.196	2.846	5.313	520 grs.
13	"	.232	2.590	2.965	5.787	730 grs.
Average,		.253	1.976	2.957	5.186	

Serum Albumin.—This is evidently more irregular in the fasting than in the normal cat. The maximum and minimum are 3.8 and 2.3 grams in the former, while in the latter they are 2.9 and 2.4 grams. The average serum albumin of the starved cats is a little higher than that of the fed ones; so that if any significance is to be attached to the small difference, starvation in this case actually increased the percentage of the serum albumin slightly.

Serum Globulin.—If there be any doubt in regard to the serum albumin, there can be none as far as the serum globulin is con-

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cerned. This suffers a decided diminution. The quantity found in any case is less than the smallest amount present in a normal cat, while the average is 42 per cent less than the normal average.

In all these respects my results agree with those of Salvioli,[12] referred to above, although they are more pronounced. This, of course, is due to the fact that his dogs fasted only about 24 hours. I find nothing to confirm Tiegel's statement.

While I agree with Salvioli[12] on the diminution in serum globulin, Howell finds it increased. In his fasting terrapin 100 ccm. of serum contained 4.7, 4.8 and 4.5 grams of serum globulin, while those in a state of active digestion only had 3.7 and 3.2 grams.

During starvation all the tissues lose weight, but some more rapidly than others. The adipose, muscular and glandular tissue are absorbed first to supply the heart and central nervous system. In this process of reabsorption the tissues must be converted again into serum albumin and serum globulin. This is evident from the fact that both continue to exist in the blood even after prolonged starvation. Why this organized material, in passing back into the blood, should in the case of cats and dogs produce serum albumin in larger quantity, while in terrapins more serum globulin is formed, is perhaps impossible to explain. But one fact seems to throw some light upon the matter.

Halliburton,[13] who analyzed the serum of a large number of cold-blooded animals, found that they all differed from warm-blooded animals in having very much less serum albumin, both absolutely and as compared with the serum globulin.

From this it would seem that while in warm-blooded animals serum albumin, if any, plays the more important part in nutrition, in cold-blooded serum globulin is the more available form of proteid. On such a supposition the different effects of starvation on the two classes of animals become intelligible, since in each that form of proteid which is normally most useful has its supply kept up while the other proteid falls off. On the other hand we have no independent evidence for any such nutritive difference between the two sets of animals as the supposition demands.

Finally, one other point which is well brought out by the analyses may be briefly alluded to, namely, the diminution of the

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total proteids by starvation. Together with the remaining tissues, the blood is included in the gradual consumption. Its loss of proteids on the average amounts to 14 per cent in fourteen days, which is considerably less than the loss of body weight. The latter, in the two cases in which it was determined, amounted to 21 per cent and 23 per cent respectively.

In conclusion, I beg leave to express my sincere thanks to Professor Martin and Dr. Howell for their kindness in placing all the facilities necessary for this work at my disposal and helping me with many valuable suggestions.

REFERENCES.

(1) Olof Hammarsten: Pflüger's Archiv, 1879 and 1880.

(2) Fredericq: Archives de Zool. Exper., tome 6. Bulletin de l'Academie Royale Belgique, 1877, t. 44.

(3) Howell: Studies from the Biol. Lab. Johns Hopkins Univ., 1884, vol. III.

(4) Mayer: Sitzungsberichte d. Wiener Acad., LVI, 1867.

(5) Bizzozero: Atti della R. Accademia d. Scienze, vol. XVI, 1881.

(6) Favilli: Archivio per le Scienze Med., vol. XIII, No. 4, 1890.

(7) Alex. Schmidt: Pflüger's Archiv, XI, 1875.

(8) Franz Boll: Du Bois-Reymond's Archiv, 1870.

(9) Tiegel: Pflüger's Archiv, vol. XXIII.

(10) N. Popoff, J. Brinck: Zeitsch. f. Biologie, VII, 1888.

(11) Burckhardt: Arch. f. Exper. Pathol. u. Pharmakol., XVI.

(12) Salvioli: Du Bois-Reymond's Archiv, 1881.

(13) Halliburton: Journal of Physiology, VII, No. 4.

(14) Howell: Journal of Morphology, vol. IV, No. 1.

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ON THE RESPIRATORY FUNCTION OF SOME MUSCLES OF THE HIGHER MAMMALIA.

By THEODORE HOUGH, A. B. With Plates IX and X.

In this paper I give the results of experiments made during April and May, 1891. My object was to determine the respiratory function of three muscles, the *external* and *internal intercostals*, and the *triangularis sterni*. The statements made in physiological text-books concerning the muscles of costal respiration are founded almost entirely upon anatomical considerations, and, as Martin and Hartwell pointed out several years ago,[1] arguments based on the points of origin and insertion of the muscles are very unsatisfactory and have led to great diversity of opinion between capable and distinguished physiologists. Especially is this true in the case of the intercostals, whose fixed point (origin) must depend on the mutual action of many other muscles acting on the ribs. It is impossible to decide *a priori* whether the anterior (cephalad) or the posterior (caudad) fixation of an intercostal muscle is to be regarded physiologically as origin or insertion; and, anatomy being quite incompetent to guide us here, the only recourse is resort to direct experiment. Moreover, in addition to determining whether a given muscle contracts during expiration or inspiration, it is important to know whether it is a muscle of normal or of forced respiration.

In 1879 Martin and Hartwell[2] studied the function of the internal intercostals in the dog and cat, by recording their contractions and relaxations on a moving surface directly under a pen which recorded the contractions and relaxations of the diaphragm. Contractions synchronous with those of the diaphragm would indicate that the muscle is inspiratory; contractions alternating with those of the diaphragm, that it is expiratory. The animals were apnœic or nearly so when the tracing was first taken, and the internal intercostal did not contract. On shutting

[1] Journal of Physiology, Vol. II, p. 24. [2] Ibid.

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off the artificial respiration, the dyspnœa which resulted was accompanied by contractions of the internal intercostals, always alternating with those of the diaphragm. This was observed in the dog at the very beginning of dyspnœa, but in the cat not until convulsions had set in. In both animals the muscle at first contracted powerfully, then seemed to become exhausted, and sometimes ceased to contract before the artificial respiration was resumed. With recovery of the animal, tracings could be obtained as before. They concluded from their experiments that the muscle is essentially expiratory. Anatomical and mechanical considerations, however, had led to a wide-spread belief that the intercartilaginous portion of the internal intercostal muscle must be inspiratory in function; and Professor Martin suggested that I should make an experimental study of the question. This led to experiments with other respiratory muscles, by the same method of taking simultaneous tracings of diaphragmatic contractions and contractions of the muscle to be studied.

My method was essentially the same as that used by Martin and Hartwell.[1] This being nearly identical in the case of all the muscles studied, a detailed description of an experiment on the right sixth *intercartilagineus* will serve to indicate the method of procedure in all cases. The animal (dog) was placed under the influence of ether, and maintained so throughout the experiment. After tracheotomy and arrangement of the apparatus for artificial respiration, a median incision through the skin and subcutaneous tissue was made the whole length of the sternum. The muscles above the intercostal fascia were reflected on the left side as far as they covered the rib cartilages. All bleeding (except in one case to be mentioned later) was stopped promptly by ligatures or hot water. The thorax was then opened anteriorly, artificial respiration properly established, and the internal mammary vessels tied. The thoracic wall was now divided on the left side near the sternum, beginning at the anterior end of the thorax and carrying the incision as far posteriorly as the seventh or eighth rib. Through the opening thus made the mediastinum was divided along its whole length and the right *triangularis sterni* removed. Next, beginning some distance dorsally and cutting toward the sternum,

[1] *Loc. cit.*

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an incision was made from the pleural side along the anterior border of the seventh rib as far as its cartilage; this incision was continued directly through the seventh cartilage, and thence along its posterior border to the sternum. A similar incision was made just posterior to the sixth rib, through its cartilage, then along the anterior border of the latter as far as the sternum. In the last operation it was impossible to avoid cutting the intercostal vein, and this bleeding had to be neglected. The overlying muscles on the right side were then dissected away, and the right ribs, from the second or third to the seventh, divided an inch or more dorsal to the union of their bony and cartilaginous portions. Finally the sixth and seventh cartilages with the muscle between were cut loose from the sternum. Thus we would have isolated a piece of the intercostal with about an inch of its two ribs attached, and connected with the rest of the body by a strip containing its artery and nerve.

The contractions of the isolated muscle and the diaphragm were recorded by means of tambours. In both cases the muscle pulled on the membrane of a *receiving* tambour, connected by rubber tubing with an inverted *recording* tambour. The receiving tambour for the diaphragm is stationary, tambour and diaphragm being connected by a string fastened to the central tendon with a hook. In the case of the isolated muscles, a serious difficulty is to keep the strip of artery and nerve still attached to the strip of muscle, from pulling on it when the ribs move in respiration, and thus, of course, vitiating the record. The method finally adopted was as follows: To a steel rod about twenty centimeters in length was fastened at one end an ordinary muscle-clamp; to the same rod was fastened also the receiving tambour some centimeters distant from the muscle-clamp. The anterior rib of the "muscle-nerve preparation" being made fast in the clamp, the posterior rib was connected by thread with the tambour, and the latter placed at such a distance from the clamp that when the muscle was relaxed the thread was taut. The whole thus formed a movable system which could be held in the hand while the tracing was being taken, and made it easy to prevent all pull on the strip of muscle. A third tambour connected with the artificial respiration pump recorded the strokes of the pump, and indicated on each tracing

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whether the artificial respiration was maintained or was temporarily stopped. In some cases a fourth pen wrote a base-line, to which the verticals of the pens are perpendicular. The pens were of course carefully adjusted to write on the same vertical on the smoked paper of the drum, or, if not, the distance between the verticals was carefully measured.

Of the muscles examined the external intercostal is the only one hard to isolate in a functional condition, possibly because of its thinness. I have kept it at work, however, in two experiments, and the tracing in Plate IX, Figure 1, from one of them gives clear evidence, if any were needed, that the muscle is inspiratory. The diaphragm pen in this tracing wrote $\frac{1}{8}$ to $\frac{1}{4}$ mm. ahead of the intercostal pen. It is evident that the external intercostal contracted exactly synchronously with the diaphragm. When the animal was made dyspnœic by shutting off the artificial respiration, the contractions of the intercostal were much increased.

Functionally the internal intercostal consists of two muscles. That portion which is covered by the external, and which we shall speak of as the interosseous internal, is expiratory, as Martin and Hartwell have shown. I have repeated their work, and my results as to this portion of the muscle agree exactly with theirs (see Plate IX, Figs. 2, 3, 4). I have also seen, as they did, the contractions become less and less toward the end of prolonged dyspnœa, although this did not take place so early as in their experiments; and during the greater part of the dyspnœa the muscle gave decidedly increased contractions. Figure 3 (Plate IX) shows this increase, and it will be noticed that there is no sign of exhaustion in the muscle, although it gave fifteen contractions since the cessation of the artificial respiration. In one case the muscle gave twenty-four contractions before becoming exhausted. The phenomenon would seem, therefore, as Martin and Hartwell have suggested, to be the result of exposure of the muscle and interference with its blood-supply.

Figure 4 (Plate IX) shows that the contraction of the interosseous internal is exactly synchronous with the relaxation of the diaphragm.

In one experiment made on the cat I was unable to get contractions of the interosseous internal intercostal until the occur-

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rence of dyspnœic convulsions, thus confirming the observations of Martin and Hartwell.

That portion of the internal intercostal muscle which is not covered by the external, and which is known as the *intercartilagineus*, has on anatomical reasoning generally been described as inspiratory in function. That this portion of the internal intercostal muscle should have exactly the opposite function from and an accurately alternating contraction with the interosseous portion, seemed so improbable that this research was started for the purpose of definitely ascertaining the fact, though other muscles were investigated in the course of it. The tracings show that the generally accepted belief is correct: the *intercartilaginei* contract in dogs at the same time as the diaphragm. Figures 5 (Plate IX) and 6 (Plate X) are tracings from this part of the internal, and show the perfect synchronism of its contractions with those of the diaphragm.

Simultaneous tracings were next taken from the diaphragm and the *triangularis sterni*. The *triangularis* of the dog arises from the lower two-thirds of the sternum; its fibres diverge to be inserted on the posterior border and inner surfaces of the costal cartilages of all the true ribs except the first and sometimes the second. The lowest fibres are at right angles to the sternum; those which succeed are oblique, while the anterior fibres are almost parallel to the sternum. In general it anatomically closely resembles the same muscle in man. Its vascular supply is chiefly through the internal mammaries, and its nerves are the intercostals.

In my experiments the muscle was left in connection with the sternum and the rib cartilages; it was also left connected with the rest of the body by a strip of tissues containing its blood-vessels, and two or more strips containing its nerves. The tracings from it were obtained by fastening the sternum in the muscle-clamp of the apparatus used for obtaining the intercostal tracings, and connecting with the receiving tambour a strip of the costal insertion of the muscle.

The anatomical relations of the muscle are such as lead to the conclusion that it must be expiratory; and experiment confirms this (see Plate X, Figs. 7, 8, 9). Its shortening begins a moment after the diaphragm has begun to relax, and ends exactly with the beginning of diaphragmatic contraction.

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When we pass to the question whether these various muscles act in ordinary or only in extraordinary respiration, we meet a more difficult problem. The cutting of so many afferent nerves, the drying and cooling of the whole thoracic region, and other causes, may act to produce abnormal breathing, even though the blood be thoroughly aerated. And it is almost impossible, by observing the animal's breathing, to say just what sort of respiration we have. As soon as the pectoral muscles are reflected, the costal attachments of the abdominal muscles cut, and the sternum removed, the movements of the ribs became vastly exaggerated; and with nothing to compare them with, we are no better able to judge from the movements of the diaphragm. If the dyspnœa have gone to the point of forcible expiration, it is, of course, easily recognized. But as to the first period of dyspnœa, marked by forced inspiration, one does not feel so sure. Perhaps the action of the second scalene would afford a clue, and in my later work I have observed whether it was forcibly contracting or not. During quiet breathing the dog's *scalenus secundus* contracts slightly, if at all; in the early stages of dyspnœa following upon cessation of artificial respiration, the movements of this muscle become very obvious. This method, however, is not satisfactory, and I shall confine myself to contrasting not ordinary and forced respiration, but inspiration slightly forced with inspiration decidedly forced.

The following tracings were taken during respiration, which if not quite normal, was but slightly dyspnœic: Plate IX, Fig. 1, from the external intercostal; Plate IX, Fig. 4, from the interosseous internal; Plate IX, Fig. 5, from the *intercartilagineus*; and Plate X, Figs. 7 and 8, from the *triangularis sterni*. The tracing from the *triangularis* in Plate X, Fig. 8, is from an experiment in which the ribs were but little moved in breathing, and in which the breathing was almost certainly normal. Moreover, in no experiment (except one on the *intercartilaginei*, when the recording apparatus was not in good working order) have I been able to stop these isolated muscles from contracting slightly at least, unless the animal was thoroughly apnœic. Either, then, the animals were always apnœic or dyspnœic (which is possible), or these muscles act in the normal breathing of the dog. The evidence is as strong that the internal intercostal and the *triangularis sterni* act in normal

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breathing as it is that the external intercostal does; and it is certain that, if these muscles do not act in quiet breathing, they are called into play with the slightest increase in the costal respiratory movements. When dyspnœa becomes pronounced, their contractions are greatly increased. In the case of the interosseous internal and of the *triangularis sterni* the following phenomenon was distinctly observed during dyspnœa: there is the first shortening, which is maintained for some time; then, just before the beginning of the diaphragmatic contraction, there occurs a still further shortening of the muscle. This is especially well marked in the *triangularis* (Plate X, Fig. 9), and continued for a time after the artificial respiration had been resumed. The animal was then probably in the expiratory stage of asphyxia, and viewed from a teleological standpoint, this supplementary contraction is clearly of use in rendering the capacity of the lungs smaller, so that during the immediately succeeding inspiration more air may be drawn into them.

As is well known, Landois and others regard the action of the intercostal muscles as accomplishing chiefly, not the raising or lowering of the ribs, but the hardening of the chest-wall during the movements of the diaphragm, so as to keep the intercostal spaces from bulging inwards or outwards during inspiration and expiration. With reference to this idea, I call attention to what appears to be a constant difference between the inspiratory and expiratory muscles. In tracings taken of the contractions of the *intercostales interossei internales* it was invariably found that at the beginning of expiration there is a more or less marked contraction, which soon reaches its maximum, and the muscle henceforth remains during expiration in a state of uniform tetanus, *i. e.*, without any further shortening or any relaxation. This fact comes out in all tracings from this muscle, and something similar, though not always so marked, is seen in tracings from the *triangularis*; here also there is at first a decided contraction, which is followed either by a more gradual shortening or by a uniform tetanus which continues to the end of expiration. Tracings from the inspiratory muscles (external intercostal and *intercartilagineus*), on the other hand, show a steady and uniform shortening continuing during the whole period of inspiration. What this differ-

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ence means I am not now prepared to discuss. It seems clear, however, that the expiratory muscles, while contracted but no longer shortening, do not continue to lower the ribs; the tense muscles would then function, as Landois has suggested, in making the chest-walls more rigid. Again, the steady, continuous shortening of the inspiratory muscles while in action must lead to a steady, continuous raising of the ribs, while, of course, also tending to render the thoracic walls more rigid.[1]

DESCRIPTION OF PLATES IX AND X.

An asterisk (*) means that the breathing was either normal or only slightly dyspnœic. In all cases *D* is the diaphragmatic tracing, and *R* that of the artificial respiration-pump. In Figs. 2, 7, 8, 9 a base-line is given for comparison of the tracings from the diaphragm and from the muscle investigated. The muscle investigated in each case is as follows:

PLATE IX.

*FIG. 1. External intercostal. The diaphragm pen is writing a little ahead of the intercostal pen (½ to ¾ mm.).

FIG. 2. Interosseous internal.

FIG. 3. Interosseous internal.

*FIG. 4. Interosseous internal.

*FIG. 5. Intercartilaginous internal.

PLATE X.

FIG. 6. Intercartilaginous internal.

*FIG. 7. *Triangularis sterni.*

*FIG. 8. *Triangularis sterni.*

FIG. 9. *Triangularis sterni.*

Each tracing is to be read from left to right. An upward stroke of the pen denotes a contraction; a downward stroke a relaxation of the muscle.

[1] It may be well to call attention to the fact that the apparent pause between the diaphragmatic movements as recorded in the tracings does not occur when the thorax is intact. Under the conditions of my experiment, not only was the rise of the diaphragm not opposed by the inflated lung, but it was actively aided by the pull of the receiving tambour.

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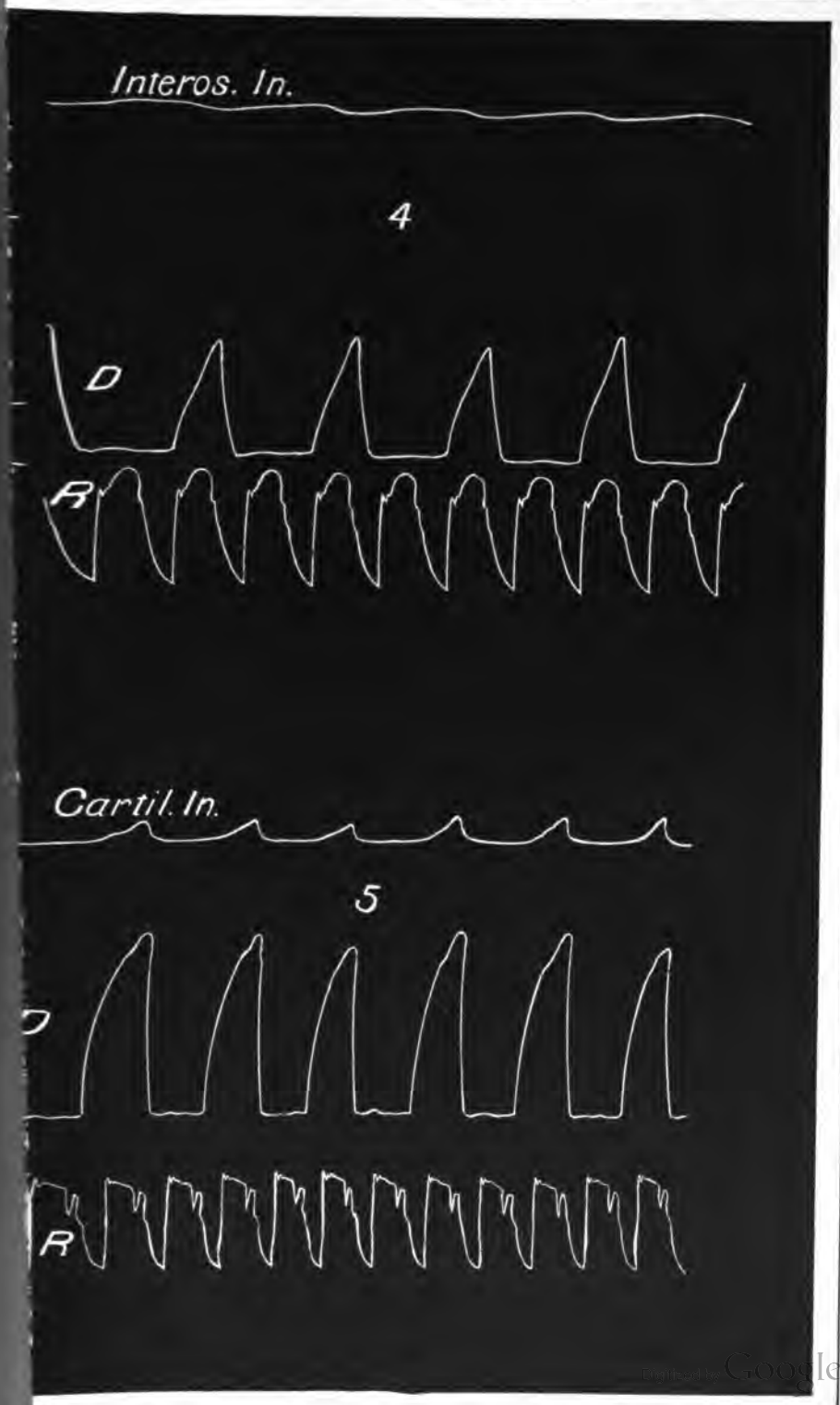

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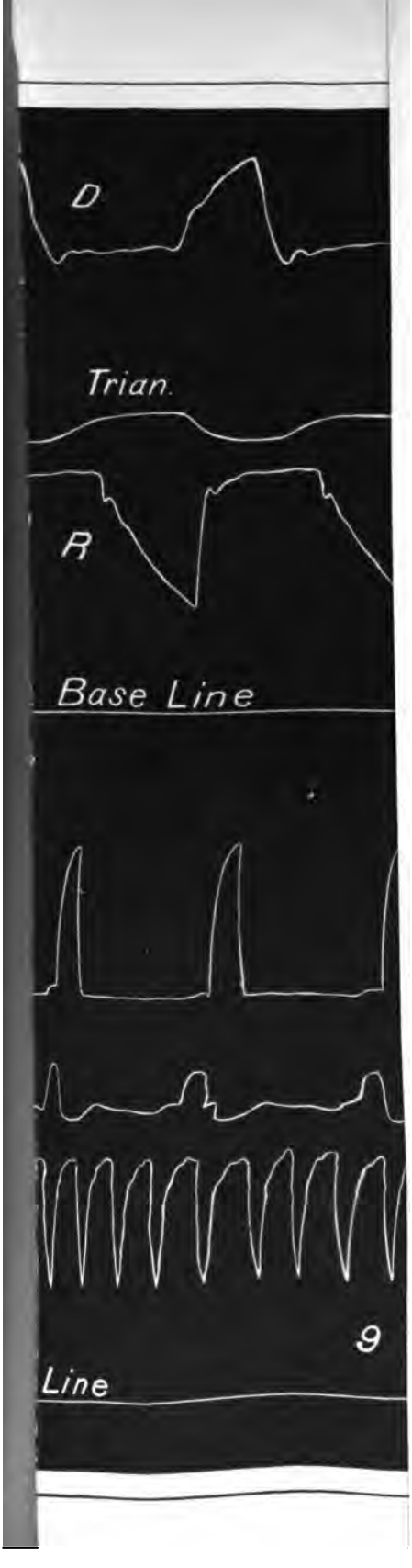

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THE LATENT TIME OF THE KNEE-JERK. By E. CAREY APPLEGARTH, B. A., Ph. D., M. D. With Plate XI.

From the time of its study by Westphal and Erb in 1875 the knee-phenomenon has been a subject of increasing interest to the physician, to physiologists, and to the psychologist. Its clinical usefulness has been especially recognized in the United States and has led to numerous investigations intended to solve questions of special interest to the physician. There is, perhaps, no other subject concerning which the scientific physician has during late years contributed so much to physiological knowledge; nor any which so well illustrates the mutual dependence of clinical observation and physiological experiment.

Most of the work in this line of inquiry has been done on man, and usually with the aim to determine the absence, presence, or amount of the knee-jerk under various conditions, normal and pathological. The accurate measurement of the *time element* is extremely difficult in human beings, and our knowledge of this element still far from satisfactory; this fact led me to the investigation now described. Without attempting here an exhaustive review of the literature bearing upon the latent time of the knee-phenomenon (or knee-jerk as many prefer to call it), a brief account of previous investigations in regard to the *time* involved will be useful.

Tschirjew[1] in 1878 undertook the solution of the problem in the following way. Over the ligamentum patellæ was bound a piece of rubber on which a bit of thin sheet copper was fastened. A conducting wire ran from this plate to an electric signal connected with a Daniell cell. From the same battery extended another wire to the metallic end of a percussion hammer, so that at the delivery of the tap upon the ligament, or rather upon the copper plate over the latter, the current was closed through the

[1] Tschirjew, Arch. f. Psych. 8, 1878, p. 689.

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signal. A contact key placed over the m. quadriceps femoris, on contraction of the muscle, directed a current from another Daniell cell through a second electric signal. Both signals, and a Marey's chronograph making one hundred double vibrations per second, wrote in the same vertical line on a revolving drum. The signals were so adjusted that their respective electrical latent periods were equal. For the results obtained by Tschirjew and others see table I, p. 102.

In the succeeding year Gowers[1] measured the knee-jerk latency by attaching to the foot a pen which traced the movement of the foot on a revolving drum, on which a chronograph pen also wrote. No mention is made of the manner of marking the instant of the tap on the ligament. Waller,[2] in 1880, caused the latent time of the knee-jerk to be "recorded by a lever-bearing tympanum, in connection with an explorer fixed on the muscle. A tube branching off the tympanum tube and crossing the tendon was first employed to record percussion. But the jar communicated to the explorer by the percussion is sufficient to record its instant." He makes use of a revolving drum, the quick rate of which was three mm. in one one-hundredth of a second. The time tracing was effected by a tuning fork making one hundred double vibrations per second.

Eulenburg[3] employed the vibrating glass plate of Landois, and Brondgeest's pansphygmograph somewhat modified. The tuning fork being struck, in the same vertical line upon the blackened plate, so set in motion, two levers traced out their respective curves, being governed by tambours provided with 90–96 cm. rubber tubing extending to two receiving tambours—one on the m. quadriceps femoris and the other upon the knee. The one fastened on the thigh was an ordinary Upham's cup, 4½ cm. diameter, bounded on the lower side by an elastic membrane with the customary button for resting upon the muscle. The one strapped around the knee, however, consisted of a brass ring, 3 cm. in diameter, over the upper and lower surfaces of which were

[1] Gowers, Med. Chir. Transactions, Vol. LXII, 1879, p. 269.

[2] Waller, Brain, July, 1880, p. 179.

[3] Eulenburg, Neurol. Centralbl. 1, 1882, S. 3; and Zeitschrift f. klinische Med. 4, 1882, S. 179.

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tied elastic membranes. In an experiment, the vibrating glass being set in motion by the assistant, the ligamentum patellæ of the subject is struck by the operator's hand; the wave of compression thus originated in the tambour covering the knee is transmitted to the recording tambour: the thickening of the muscles, which contract to produce the knee-jerk, initiates from the Upham's cup a similar chain of events; and the latent time of the contraction may at once be read off in swings of the tuning fork. The time (0.003″) taken by the wave to traverse the tubing connecting the receiving with the recording tambours is eliminated by having the same length of tubing in each case.

Rosenheim[1] attacked the problem with an instrument specially devised for the purpose. It consists of two uprights, one immovably attached near its upper extremity to the bottom of a V-shaped collar, and the other mobile around a Charnier's joint in the two arms of the latter. A little above this joint there is on the one upright a plate for making electrical contact with a screw carried by the other. The uprights may be adjusted by means of a spring situated below the joint. On the lower edge of each upright is a ring, to which is secured the band strapping the instrument to the leg. When the muscle contracts, contact is broken between the screw and the corresponding plate, and an electric signal marks this instant on a revolving drum, whose quick rate equals one metre of paper in thirteen seconds. The tap upon the patella is registered by another signal, the action of which depends upon a blow from a percussion hammer in connection with a Daniell cell, upon a piece of copper or tin foil, also in connection with the battery, and placed over the ligament.

The results of these investigators along with those of A. de Watteville,[2] who availed himself of Waller's method, and those of Brissaud,[3] who used an electric signal for recording the tap, and a Mendelssohn's myograph to record the contraction of the muscle, may be tabulated as follows:

[1] Rosenheim, Arch. f. Psychiatrie, XIV, 1884, S. 184.
[2] A. de Watteville, Brain, July, 1882, Vol. 5, p. 287.
[3] Brissaud, Publications du Progrès Med., Paris, 1880, p. 206.

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TABLE I.

Worker.	Subjects.	Observations.	Latency.
Tschirjew,	2 patients.	146	0.0595″
Gowers,	2 normal persons. 1 case of lateral sclerosis.	?	0.100
Waller,[1]	12 patients.	?	0.035
Brissaud,	? normal. ? hemiplegia.	?	0.050 0.015
Eulenburg,	100 students.	?	0.024
A. de Watteville,	1 patient.	?	0.025
Rosenheim,	4 individuals.	?	0.04313
	cases of hemiplegia.	?	0.025
	10 rabbits normal.	?	0.033
	10 rabbits brain excluded.	?	0.023

With the exception of that of Gowers, it will be seen that in all the above contributions to the literature of the subject in hand, the contraction of the m. quadriceps femoris has been recorded by implements which depend for their efficiency upon the swelling of the muscle as it contracts. Remembering how difficult it is, even with an isolated muscle, to obtain apparatus sufficiently delicate to render manifest the initial stage of contraction, and having in mind the importance of the question as to whether the knee phenomenon is or is not a reflex action, it seemed desirable to make further experiments with more accurate methods.

I have chosen dogs as the subjects of my research. In a later paper I hope to discuss the matter more fully and to give observations on the human subject.

In the present investigation five hundred and eighty experiments were made on thirty-two dates upon three bitches, which for the sake of convenience may be designated A, B, C. Of these experiments, one hundred and one were performed upon A, two hundred and ninety-one on B, and one hundred and eighty-eight on C. A and B had their spinal cords severed at the level of the last dorsal vertebra, C was normal. All three were small and as similar as it was possible to obtain them.

[1] Since writing the above I notice Waller has published a more recent research in the Journal of Physiology, Vol. XI, p. 384. With improved apparatus he finds that 0.012″ intervenes between the instant of percussion upon a rabbit's tendon and the resulting muscular contraction. Deducting 0.005″ for the mechanical lost time, and adding 0.001″ to counterbalance the lost time of the Despretz signal, he obtains the corrected interval 0.008″.

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A was operated upon with aseptic precautions during the first week of April, 1889. She recovered well and remained strong and healthy; but owing to other duties it was impossible for me to experiment upon her knee-jerk until the following January. Towards the latter part of February (1890), in consequence of an attack of mange, a lotion was applied to her skin, which she licked off, poisoning herself. The autopsy, made with the kind assistance of Dr. W. T. Councilman of the Johns Hopkins Hospital, showed the severed spinal cord was reunited by dense fibrous tissue. Beyond obvious degeneration of certain nerve tracts (which had been going on for eleven months prior to death) no pathological change was manifest in the spinal cord. The whole cerebro-spinal axis was carefully preserved for future microscopical examination.

As the cord of A was cut above the centres of defecation and micturition, there was no *à priori* reason why these natural actions should not be reflexly excited, and this conclusion was amply justified by the facts. As Goltz found, sponging the anus, pinching the tail, and even the dragging of the hinder parts about by the animal itself while it crawled on its fore limbs, proved effectual in leading to evacuation of the bladder and rectum. Whenever micturition or defecation occurred there was an accompanying train of reflexes involving the muscles of the tail and hind legs.

The animal was fed and exercised with great regularity. Her exercise consisted in permitting her, while I supported her hind limbs off the floor, to run or walk where and how she liked for fifteen to thirty minutes in the morning, the same at noon, and one-half to one hour at night. She was always at liberty, when not at exercise, and could crawl around as much as she pleased. In addition the muscles of the paralysed legs were exercised by electric stimuli, and from the date on which the knee-jerk observations commenced almost daily experimentation added its quota towards keeping the muscles in healthy condition. The expenditure of time and energy involved in caring for this animal during so many months was very great, but in no other way can good health be secured to a dog with severed spinal cord, and

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the good health of the animal's muscles and nerves was essential to me.

B was operated upon the day following the death of A, and was used for experimentation upon her knee-jerk two weeks later. Her health remained good. The same care was taken as with A.

C, as stated above, was normal, and as far as possible kept under the same conditions as were A and B.

All three dogs were young, and were taught without much difficulty to lie quiet when experimented on.

A thin cushion placed on a board formed a bed. From one side of the boards two straps extended. One strap passed over the neck and the other over the body just posterior to the forelegs. This bed was not disagreeable; indeed, the dogs appeared to find it comfortable, since they would often go to sleep on it.

While the trunk was thus kept quiet the leg (in all cases the right) whose knee-jerk was to be investigated was held in a large retort-holder which could be adjusted to fit snugly, binding upon the condyles of the femur with pressure just sufficient to hold the leg securely. The upright carrying this holder was clamped to the table, so that the only movement that could take place, when the ligamentum patellæ was struck, was that of the tibia on the knee-joint. The foot was strapped in two places, not too firmly, to a splint extending from the toes to a point a little past the tarsus.

This splint is of wood one inch thick, three long, one broad, and has its upper surface concave. At each end there are two buttons, one on each side, for leather straps hanging from a hook which swings in an iron thimble at the end of a cord suspended from the ceiling. At the distal end of the splint is a binding screw, into the top of which is soldered a stiff platinum wire. The latter may be made to press against a contact plate of platinum that is fastened upon a block of vulcanite, which in turn slides upon an upright similar to that of a Roy's universal holder. A Pfeil signal is included in this circuit.

Over the ligamentum patellæ was attached, by means of adhesive plaster, a thin narrow strip of brass covered with platinum. Upon this strip of brass, which also stands in electrical connection with a Pfeil signal, impinges a hammer the exact duplicate of

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Lombard's,[1] with the exception that the head is very much lighter, weighing only ten grams, and is insulated from the handle, and that instead of the catch holding the head up is an electro-magnet performing the same office. This hammer, being in the same circuit as the strip of brass fitting snugly over the knee, causes, when it strikes the latter, a closure of the current, which instant the signal records. The contact edge of the head of the hammer is covered with platinum, as in all other cases where instantaneous electrical connection was desired. Throughout the whole research a very light tap was used, due to a fall of the hammer of two degrees of the circle.

The exact arrangement of these various parts can best be understood by reference to the diagram represented in Fig. 3, Plate XI. *B* is a battery of two large Daniell cells, while *T* is a break mechanism or trigger like that of a pendulum myograph. When *T* is up against the screw, and *H* is not touching *K*, the current finds a complete circuit through the electro-magnet *M* back to the battery. When, however, the trigger is down, as shown in the diagram, the current can no longer flow through *M*, but is compelled to pass through *H*, the hammer, *K*, the knee piece, *S*, the signal, and thence back to the battery. But this circuit is only closed when *H* touches *K*.

Fig. 2, Plate XI, shows in a diagrammatic way the circuit of the other signal. *B* is a battery of one large Daniell cell. *P* is the stiff platinum wire making contact with *C*. When *P* is pressing upon *C* the current flows from the battery to *P*, through the contact plate *C* and back to the battery. When, however, contact is broken between *P* and *C* the current must pass through *wr*, which is of very fine wire, to the signal *S*, and back to the battery. *K* is simply a key which opens this circuit when desired.

The signals, as mentioned above, are of the same make; they write in the same vertical line on the smoked glass plate of the pendulum myograph described by Sewall,[2] and are placed upon a supporting table which can be screwed up until the writing points of the levers just touch the blackened plate. The latent period

[1] Lombard, Amer. Journal of Psychology, Vol. I, 1887, p. 5.
[2] Sewall, Jour. Physiol., Vol. II, p. 164.

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of each was carefully traced on the myograph plate, which for the purpose was made to swing through an arc of displacement 646.8 mm., with therefore a velocity of 1 mm. in 0.000492″ as it passes the writing point of the signal. The difference in the time equivalents of the signals was found to be 0.00008″.

After trying fish-bones, glass drawn out to a very fine point and other objects, I finally decided on exceedingly delicate cambric needles as most satisfactory for the writing points.

The pendulum myograph in use in our laboratory beats true seconds, and swung, in the present inquiry, through an arc of displacement of 220 mm. Consequently if v equals the velocity of the centre of the plate as it passes the zero point of fixed scale corresponding to the middle of the swing of the pendulum, ch the chord of the arc of displacement of the centre of gravity of the pendulum, g the intensity of gravity, and l the length of a second's pendulum at the place in question, then the formula

$$v = ch\sqrt{\frac{g}{l}}$$

gives at once the desired information. In this way the rate was calculated to be 1 mm. in 0.001446.″ As a matter of fact it was found that this rate did not measurably vary for the first two centimeters on each side of the central line of the plate, but nevertheless a tuning fork making 200 double vibrations per second was from time to time employed as a control.

The actual order of each experiment has been as follows: The dog having been placed on the bed, its leg is clamped at the knee and its foot strapped to the splint. After the foot has come to its position of rest, the contact plate *C*, Fig. 2, Plate XI, is pushed up against the stiff wire *P* on the splint until the foot is moved forward about an inch, so that considerable pressure is thereby exerted by the wire upon the contact plate. When so arranged, moderate blows on the splint and distal portion of the tibia prove ineffectual in breaking contact. When, however, the hammer excites the knee-jerk by striking on the ligamentum patellæ, contact is broken and the latency of the phenomenon is registered.

I hoped by thus taking advantage of the great leverage of the extensors of the crus to be able to obtain the record of an early, if not the earliest, stage of contraction.

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The trigger *T*, Fig. 1, Plate XI, slides upon the metal micrometer scale of the myograph, and is so placed that when the latter in its swing knocks it, the signal marks near the middle of the plate the instant of the ensuing tap upon the tendon. This position having been secured, all is ready for an experiment.

The following tables give details of a few of the experiments performed in this way. $R_1 - R_2$ represents the distance expressed in mm. from the beginning of the tracing of one signal to the like point of the other's tracing. The second column contains the time equivalents of the given number of mm. (= mm. $\times$ 0.001446″), and the third column the equivalents corrected for difference in latency of the two signals (= [mm. $\times$ 0.001446″] + 0.00008″).

TABLE II.

Jan. 30, 1890. Bitch A. Cloudy. External temperature 9° C. Room temperature 20° C. Dog exercised but not fed. Began 9.30 A. M. Observations made every minute.

$R_1 - R_2$.	Time.	Corrected Time.
5.8	0.00766″	0.00774″
7.2	0.01041	0.01049
9.0	0.01301	0.01309
7.2	0.01041	0.01049
7.8	0.01128	0.01136
7.1	0.01027	0.01035
8.4	0.01215	0.01223
7.3	0.01056	0.01064
9.0	0.01301	0.01309
5.7	0.00824	0.00832
8.8	0.01272	0.01280
6.4	0.00925	0.00933
5.8	0.00839	0.00847
8.8	0.01272	0.01280
6.1	0.00882	0.00890

Average, 0.01060″
Corrected average, 0.01068″

TABLE III.

March 25, 1890. B. Cloudy. External temperature 3.5° C. Room temperature 25° C. Dog exercised but not fed. Began 9.15 A. M. Observations made every minute.

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$R_1 - R_2$.	Time.	Corrected Time.
9.5	0.01374″	0.01382″
6.5	0.00940	0.00948
7.8	0.01128	0.01136
7.2	0.01041	0.01049
7.9	0.01142	0.01150
6.0	0.00868	0.00876
9.8	0.01417	0.01425
8.2	0.01186	0.01194
9.4	0.01359	0.01367
8.8	0.01272	0.01280
7.9	0.01142	0.01150
7.4	0.01070	0.01078
7.5	0.01085	0.01093
6.8	0.00983	0.00991
6.1	0.00882	0.00890
6.8	0.00983	0.00991
6.4	0.00925	0.00933
8.2	0.01186	0.01194
6.1	0.00882	0.00890
	Average, 0.01098″	
	Corrected average, 0.01106″	

TABLE IV.

March 24. C. Clear. External temperature 6° C. Room temperature 23° C. Dog exercised but not fed. Began 12 M. Observations made every minute.

$R_1 - R_2$.	Time.	Corrected Time.
7.2	0.01041″	0.01049″
6.9	0.00998	0.01006
9.0	0.01301	0.01309
9.8	0.01417	0.01525
9.1	0.01316	0.01324
9.6	0.01388	0.01396
10.4	0.01504	0.01512
8.9	0.01287	0.01295
9.5	0.01374	0.01382
8.8	0.01272	0.01280
10.1	0.01460	0.01468
8.0	0.01158	0.01166
9.2	0.01330	0.01338
10.2	0.01475	0.01483
8.3	0.01200	0.01208
10.5	0.01518	0.01526
9.2	0.01330	0.01338
9.5	0.01374	0.01382
	Average, 0.01320″	
	Corrected average, 0.01328″	

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After doing a number of experiments in this way it occurred to me to test these results by causing the knee-jerk to make contact. Of course some time must be lost hereby, but nevertheless the averages should be approximately equal, *caeteris paribus*. The only change in the apparatus necessary for this purpose was to dispense with the resistance circuit *wr*, Fig. 2, and put the signal in the primary circuit. Now the contact plate *C*, Fig. 2, is placed in front of *P* at a distance of 1 mm., and an upright is clamped to the table back of the splint, so that the leg may have exactly the same amount of flexion at the beginning of each experiment. The following tables give a few of the protocols so obtained.

TABLE V.

March 19, 1890. B. Cloudy. External temperature 5° C. Room temperature 22° C. Began 10 A. M. Observations every minute.

R_1-R_2.	Time.	Corrected Time.
9.8	0.01417″	0.01425″
9.6	0.01388	0.01396
9.2	0.01330	0.01338
9.5	0.01374	0.01382
10.5	0.01518	0.01526
5.0	0.00723	0.00731
8.0	0.01157	0.01165
5.2	0.00752	0.00760
6.1	0.00882	0.00890
8.8	0.01272	0.01280
12.5	0.01806	0.01814
9.0	0.01301	0.01309
5.0	0.00723	0.00731
5.8	0.00839	0.00847
7.6	0.01099	0.01107
8.0	0.01157	0.01165
10.2	0.01475	0.01483
	Average, 0.01189″	
	Corrected average, 0.01197″	

TABLE VI.

March 20, 1890. C. Cloudy. External temperature 7° C. Room temperature 22° C. Began 2 P. M. Observations every minute.

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R_1-R_2.	Time.	Corrected Time.
17.5	0.02521″	0.02529″
10.6	0.01533	0.01541
12.0	9.01735	0.01743
13.0	0.01880	0.01888
9.0	0.01301	0.01309
8.8	0.01272	0.01280
8.5	0.01230	0.01238
9.8	0.01417	0.01425
13.6	0.01967	0.01975
11.0	0.01590	0.01598
16.5	0.02386	0.02394
	Average, 0.01712″	
	Corrected average, 0.01720″	

Desiring now to apply the method depending upon the thickening of the muscle as it contracts, a piece of brass three mm. square was bound over the m. quadriceps femoris by an elastic band. Soldered to this plate were a copper wire, leading to the battery, and a stiff platinum wire, extending vertically upwards. Near its free end the latter was bent at right angles, and by means of a micrometer screw a sliding stage could be raised up under the horizontal arm until firm yet delicate contact was made. The connections with the stage working on the micrometer screw were the same as with *P*, Fig. 2, so that, as soon as the muscle contracted, the circuit was opened and the signal recorded. Moderate shocks on the splint and body did not suffice to break contact.

The following are some of the results furnished by this method.

TABLE VII.

March 21, 1890. B. Cloudy. External temperature 12° C. Room temperature 22° C. Fed at 2 P. M. Began 5 P. M. Observations half a minute apart.

R_1-R_2.	Time.	Corrected Time.
12.5	0.01808″	0.01826″
11.8	0.01706	0.01714
6.4	0.00925	0.00933
7.0	0.01012	0.01020
7.5	0.01085	0.01093
7.2	0.01041	0.01049
14.9	0.02155	0.02163

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R_1-R_2.	Time.	Corrected Time.
11.5	0.01668″	0.01671″
8.8	0.01272	0.01280
9.2	0.01330	0.01338
8.8	0.01272	0.01280
16.0	0.02314	0.02322
12.4	0.01793	0.01801
9.5	0.01374	0.01382
9.8	0.01417	0.01425
9.6	0.01388	0.01396
10.2	0.01475	0.01483
11.1	0.01605	0.01613
10.2	0.01475	0.01483
10.5	0.01518	0.01526
10.1	0.01460	0.01468
11.0	0.01591	0.01599
13.5	0.01952	0.01960
9.1	0.01316	0.01324
10.0	0.01446	0.01454
9.2	0.01330	0.01338
9.9	0.01432	0.01440
10.5	0.01518	0.01526
6.5	0.00940	0.00948
8.8	0.01272	0.001280
9.6	0.01388	0.01396
7.6	0.01099	0.01107
9.0	0.01301	0.01309
9.5	0.01374	0.01382
10.2	0.01475	0.01483
9.8	0.01417	0.01425
	Average, 0.01443″	
	Corrected average, 0.01451″	

TABLE VIII.

March 22, 1890. C. Rain. External temperature 10° C. Room temperature 22° C. Fed 12 M. Began 5.30 P. M. Observations every minute.

R_1-R_2.	Time.	Corrected Time.
10.4	0.01504″	0.01512″
14.6	0.02111	0.02119
13.5	0.01952	0.01960
15.2	0.02298	0.02306
9.0	0.01301	0.01309
15.5	0.02241	0.02249
16.8	0.02429	0.02437
13.0	0.01880	0.01888

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R_1-R_2.	Time.	Corrected Time.
14.8	0.02140″	0.02148″
15.0	0.02169	0.02177
9.9	0.01432	0.01440
13.0	0.01880	0.01888
13.3	0.01923	0.01931
14.2	0.02053	0.02061
11.2	0.01620	0.01628
	Average, 0.01928″	
	Corrected average, 0.01936″	

The results by averages of all the experiments by each method may be tabulated as follows: By the first method where the knee-jerk caused interruption of the electric current the average latent time in case of A and B was 0.010″, while with C it was 0.014. By the second method above given the average for B was 0.015″, while for C 0.02. By the third method where the registration of the signals depended upon the thickening of the muscles, the average latent time for B was 0.016″, while for C it was 0.020.

From a comparison of these results it seems probable that the latency of the knee-jerk of the dog, as obtained by the electrical method, is not over 0.014″–0.020″.

In applying the second method, as above given, it was frequently noticed that if the distance between *C* and *P*, Fig. 2, Plate XI, was over 1 mm., say 1½–2 mm., the corresponding difference in time became excessively disproportionate, in fact almost equivalent to the time of a superficial reflex. The following table will illustrate this.

TABLE IX.

March 20, 1890. C. Cloudy. External temperature 5° C. Room temperature 22° C. Exercised and fed. Began 2 P. M. Observations made every minute. Distance of *C* from *P* 2 mm.

R_1-R_2.	Time.	Corrected Time.
34.9	0.05047″	0.05055″
33.6	0.04867	0.04875
40.9	0.05914	0.05922
42.8	0.06189	0.06197
38.2	0.05522	0.05530
43.2	0.06247	0.06255
41.0	0.05929	0.05937
41.4	0.05986	0.05994
40.2	0.05813	0.05821
	Average, 0.05724″	
	Corrected average, 0.05732″	

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whereas, the average of a number of time measurements of skin reflexes elicited by electrical stimulation applied to the toe of A was 0.07141″. This at once reminds us of what Gowers observed, namely, that when the ligamentum patellæ was struck the curve traced by the foot left the abscissa very soon, but the true contraction of the muscle followed at a somewhat later period.

It will be seen also that by each method the latency of the knee-jerk is less in the dogs with their spinal cords cut than in the normal one. This is in keeping with results previously obtained, and tends to confirm the view that the brain is one of the important factors concerned in the time of the phenomenon.

Lombard found the extent of the knee-jerk was very profoundly influenced by the emotions, variations of weather and the like, and it seemed of interest to me whether such changes involved corresponding differences in the time. In this respect, however, my results were not quite satisfactory, and after the first few experiments it appeared best to defer this matter for the present.

In conclusion, I desire to acknowledge my obligations to Professor H. Newell Martin for his kindness and valuable suggestions.

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SOME EXPERIMENTS AS TO THE PHYSIOLOGICAL EFFECTS OF "DIFFERENTIAL RESPIRATION."

By H. NEWELL MARTIN, M. A., M. D., F. R. S., and G. P. DREYER, B. A., Ph. D. With Plate XII.

Some years ago there was put before the medical profession a method of treating pulmonary diseases, especially phthisis, by the use of a so-called "*pneumatic cabinet.*" This cabinet was so arranged that while the patient breathed ordinary atmospheric air under normal atmospheric pressure, the air which surrounded him could be rarefied or compressed at will, and thus an abnormal expansion or an abnormal compression of the thoracic and abdominal cavities be brought about with each respiration. The name "differential respiration" was given to this process, and it was widely advertised by the patentee of the "cabinet." What the physiological effects of breathing under such very unusual conditions might be seemed a question of sufficient importance to justify a careful experimental investigation.

The phenomena produced by breathing rarefied air and by breathing compressed air had been studied, but here was a new problem, namely, the results of breathing air under normal pressure while the whole surface of the body was exposed to air under abnormal pressure. One of us, soon after the "pneumatic cabinet" was put on the market, made some experiments in conjunction with Dr. F. Donaldson, Jr.,[1] which proved that the pneumatic cabinet should be used with great caution, since when the air in it was only moderately rarefied, fainting was produced in healthy rabbits. Dr. Donaldson's departure from the city brought the investigation to a close before any thorough research had been made, and in this paper we give some of the results of a prolonged research which is not yet concluded. For the present we shall deal only with the effects of rarefaction of surrounding air on an animal breathing air under normal pressure.

The pneumatic cabinet supplied to physicians was unnecessarily heavy and cumbrous for use with rabbits and cats, so we had to

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plan something more suited to our purpose. To get a perfectly air-tight box we found much more troublesome than we had anticipated, but we finally devised the apparatus described below, which has proved quite satisfactory.

It consists of a rectangular iron box 90 cm. long, 40 cm. high and 35 cm. wide, completely open on one of its long sides which will be spoken of as the top. It can therefore be cast in one piece, as was the case with our box whose walls are somewhat less than 1 cm. thick.

The free edge of the box extends horizontally outwards to the extent of 3 cm. in the form of a ledge running around the four sides, the upper surface of this ledge being planed so as to fit accurately against a corresponding surface of the lid of the box to be described presently.

Over an area of about 15 cm. square in the middle of one end of the box the wall is made two or three times as thick as elsewhere, and here a number of openings are drilled to establish communication with the outside.

A detailed description of the lid which is necessay to complete the cabinet is impossible without the use of diagrams. Suffice it to say that it too was made of cast-iron, but instead of a continuous sheet of metal, consists only of a frame which may be compared to a window-sash divided by two cross-bars into three sections, each of which is occupied by a pane of heavy plate glass. In this way light may penetrate the box and enable one to observe the animal during the course of an experiment.

The frame of the lid presents a flat planed under-surface, with which it rests on the upper surface of the ledge of the box spoken of above. Between these two surfaces, moreover, there is placed a sheet of pure rubber ½ cm. thick and 3 cm. wide, effectually preventing all leakage at this point when the lid was screwed down. For this purpose strong bolts were used at intervals of 15 cm. around the four sides, the bolts passing from below upwards through the ledge of the box, the rubber pad and the cover. The square heads of the bolts prevent them from turning when the nuts are tightened up by hand. To close the box, therefore, after the animal is introduced, the lid is placed in position, the bolts to the number of about 20 inserted and the nuts screwed on.

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This method of closing the box may seem very objectionable at first sight on account of the apparently great expenditure of energy and time which it involves. In reality, however, one soon learns to do it rapidly and without much trouble; we found that it took us only from 2 to 3 minutes, as a rule, to put the animal in the box, make all the necessary connections and screw down the lid. Moreover, in most of the experiments, when once closed, the lid remains in position until the experiment is finished. Where the nature of the experiment requires frequent opening of the box it becomes a more serious matter. More convenient methods for fastening down the lid were tried, but none of them succeeded in rendering the box completely air-tight.

A number of openings in one end of the box were spoken of above. By means of one of these holes communication is established between the outer air and the trachea of the animal, in this way supplying air under normal atmospheric pressure after closure of the box, at the same time providing the means for continuing the application of ether when desired. A second opening was required for the connection between the blood-pressure manometer of Ludwig's kymograph and the carotid artery. The interior of the cabinet communicated by another opening with a second mercury manometer, by means of which a continuous record of the air-pressure in the box throughout the experiment is obtained on the kymograph paper (see Pl. XII, *MN*). Finally the cabinet was connected with a three-way stopcock, by means of which its interior could be put in communication on the one hand with the outside air, or else with an air-chamber of about 75 litres capacity. This reservoir was exhausted with an air-pump before the experiment began, and so enabled us by simply turning the stopcock to rarefy the air in the cabinet quickly to the desired extent. On reversing the stopcock the air rushed into the cabinet again, and this process could be repeated a number of times. The pens of the two manometers already mentioned, and a time-marker indicating seconds, are arranged so as to write in the same vertical line.

The experiments described below were made on etherized cats, ether being an anæsthetic which could be easily renewed during the experiment whenever necessary, on account of the communication of the trachea of the cat when in the box with the exterior as

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described above. It has already been mentioned that the blood pressure was taken from the carotid artery, especially because it was intended to cut the pneumogastrics in many cases, and one operation served both purposes.

In general, the anæsthetized animals stood the experiment so well that the rarefaction could be repeated six or more times.

We give below in tabular form the detailed results of six experiments, as a basis for discussion as to the general outcome of our work.

TABLE I.

Time. H.	M.	S.	Pressure in Case.	Blood Pressure.	Pulse.	Cat.
8	9	50	Normal.	177 mm.	267	II.
	10		Rarefaction of 1.2 cm. of mercury.	136	282	"
		10		146	282	"
		20	Return to normal.	166	255	"
	11	40	Normal.	160	248	"
		50	"	160	244	"
	12		Rarefaction of 3.4 cm.	58	255	"
		10	" "	130	264	"
		20	" "	98	264	"
		30	" "	111	249	"
		40	" "	105	246	"
		50	" "	111	244	"
	18		" "	114	284	"
2	59	40	Normal.	137	231	III.
		50	"	137	228	"
3			Rarefaction 1.5 cm.	80	(?)	"
		10	" "	124	261	"
		20	" "	124	249	"
		30	" "	127	252	"
		40	" "	128	255	"
		50	" "	128	252	"
	1		" "	133	240	"
		10	" "	135	231	"
		20	" "	137	219	"
		30	" "	140	210	"
3	53	10	Normal.	142	185	XXII.
		20	"	142	189	"
		30	Rarefaction 1.4 cm.	82	192	"
		35	" "	122	228	"
		40	" "	130	204	"
		45	" "	113	192	"
		50	" "	103	180	"
		55	" "	99	180	"
	54		" "	96	168	"

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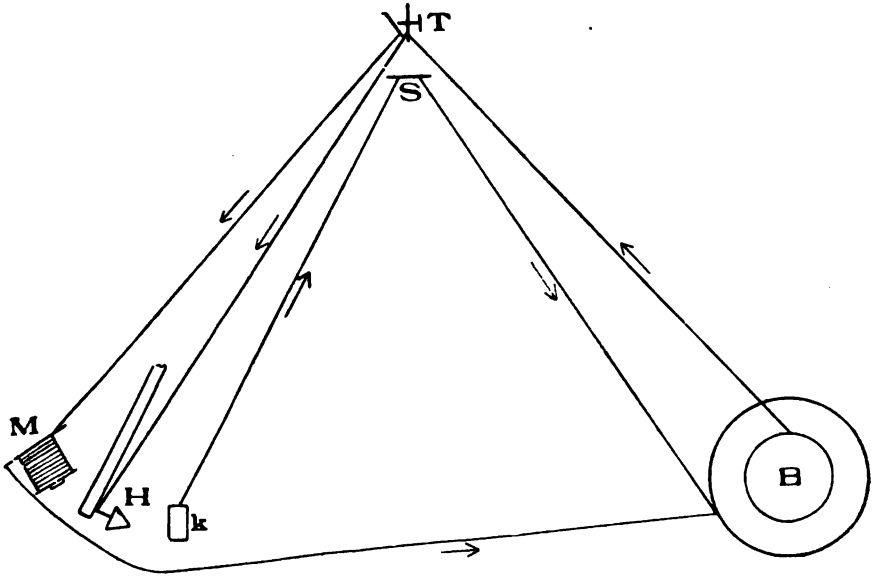

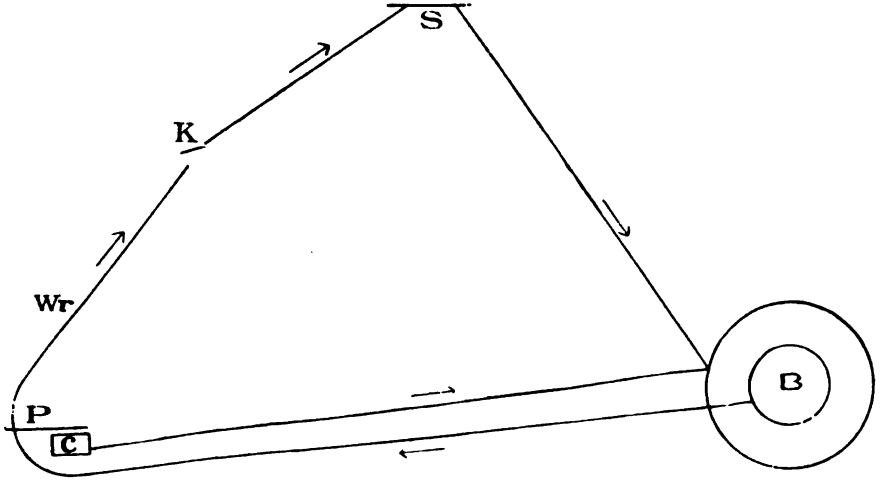

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Time. H.	M.	S.	Pressure in Case.	Blood Pressure.	Pulse.	Cat.
3	21	40	Normal.	210	234	XXIV.
		50	"	208	231	"
	22		Rarefaction 1.4 cm.	145	252	"
		5	" "	141	240	"
		10	" "	138	240	"
		15	" "	126	228	"
		20	" "	120	240	"
		25	" "	113	222	"
		30	" "	105	217	"
		35	Return to normal.			
	26	40	..	160	174	"
		50	..	157	168	"
	27		Rarefaction .6 cm.	116	168	"
		5	.. ..	117	180	"
		10	.. ..	118	180	"
		15	.. ..	118	180	"
		20	.. ..	118	180	"
		25	.. ..	117	168	"
		30	.. ..	115	168	"
2	11	40	Normal.	148	192	X.
		50	"	147	195	"
	12		Rarefaction 2.7 cm.	24	237	"
		10	" "	86	231	"
		20	" "	72	212	"
		30	" "	69	210	"
		40	" "	78	213	"
		50	" "	95	210	"
	19	40	Return to normal.	129	168	"
		50	"	127	168	"
	20		Rarefaction 5.7 cm.	40	no pulse.	"
		10	" "	..	..	"
		20	" "	..	..	"
		30	Return to normal.			
		40	"	70	188	"
		50		136	207	"
				etc.	etc.	
3	58	45	Normal.	145	177	XXVI.*
		50	"	144	180	"
		55	"	144	174	"
	54		Rarefaction 1.2 cm.	80	183	"
		5	" "	83	180	"
		10	" "	84	180	"
		15	" "	81	180	"
		20	" "	80	174	"
		25	" "	80	171	"
		30	" "	81	171	"
		35	" "	82	171	"
		40	" "	83	174	"
		45	Air let in.	136	171	"

* This portion of the table was compiled from the tracing given in Plate XII.

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The table shows that the amount of rarefaction employed varied considerably; the extremes being 5 mm. and 5.5 cm. of mercury pressure.

No graphic record was taken of the respiratory movements of the animal, but direct observation showed that in nearly every case a rarefaction equaling 1.5 cent. mercury pressure caused a resistance that the expiratory muscles could not overcome. When the air surrounding the animal was rarefied to this extent, expiration became impossible, the muscles could not overcome the resistance opposed to them.

Rarefaction exceeding 1.5 cm. of mercury pressure caused a deep initial inspiration, and then the thorax remained expanded as long as the rarefaction was maintained: the animal was in a condition of *mechanical* apnœa.

The tables also make it clear that a difference of but six mm. of mercury between the intra-pulmonary and extra-thoracic pressures is sufficient to set up marked circulatory disturbances, involving both arterial pressure and pulse-rate. The variations in arterial pressure due to rarefaction of the air were always similar, though varying much in degree. *Whenever extra-thoracic atmospheric pressure is lessened, blood pressure falls,* if the lungs be still supplied with air under normal atmospheric pressure.

This is illustrated by many cases given in the foregoing table, *e. g.*:

Rarefaction.	Arterial Pressure	
0.6 cent. Hg	falls	40 mm. of Hg.
1.4 " Hg	"	60 " "
2.7 " Hg	"	120 " "
5.7 " Hg	"	177 " "

In the last of the above cited examples, arterial pressure in the carotid was 40 mm. below the zero or base line of the manometer, indicating an actual suction of blood from the systemic arteries, causing a negative pressure in them.

As a rule partial recovery takes place from the first sudden fall of arterial pressure, but this is but transient and never brings arterial pressure back to what it was before the rarefaction; and during the rarefaction, continued in some cases for four minutes, arterial pressure always remained below the normal.

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In Plate XII is given a copy of a tracing showing the effect of extra-thoracic rarefaction on arterial pressure: the sudden fall, temporary recovery, and the final permanent lowering.

In attempting to explain this great and sudden fall of pressure we had to consider observations by Paul Bert and recorded in his well known book "*Pressions Barometrique.*" Bert states that at high altitudes attained quickly, as by mountain-climbing or a balloon ascent, superficial veins (those of the eyelids and lips, for example) become greatly congested, and that bleeding from the nose and eyes is apt to occur. The thin-walled and slightly muscular veins respond more quickly to diminution of external pressure than the thicker walled, elastic and muscular arteries, and so the veins become gorged while the arteries empty. Such a filling of the venous system at the expense of the arterial must bring about a fall of arterial pressure, unless there were compensating constriction of the arterioles.

In Bert's work the diminished atmospheric pressure influenced equally the pulmonary and systemic vessels, which was not the case in our experiments. But it is obvious that diminution of atmospheric pressure on the surface of the body while intra-pulmonary air pressure remained normal would tend still more strongly to cause dilatation of superficial veins and capillaries. But such diminutions of atmospheric pressure as we used (a few millimeters or centimeters of Hg) are hardly comparable with those experienced by Glaisher and others in balloon ascents. Such a fall of arterial pressure as occurs in the "pneumatic cabinet" with a slight rarefaction of the air would be fatal to balloonists long before they reached such altitudes as those recorded by Glaisher and others; altitudes at which the barometric pressure was far lower than in any of our experiments. We found that the blood was drawn from the systemic arteries as indicated by fall of carotid pressure, and we could not account for the whole or even a large proportion of it, as gathered into the veins of the skin. Our first problem was, where did the blood go when it had been so markedly abstracted from the systemic arteries?

Our first idea was that this blood might be stored in the vessels of the abdominal viscera. The thorax and skull being relatively

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unyielding, and the belly walls soft, it was easy to conceive that when pressure on the exterior of the animal's body was decreased there would be an accumulation of blood in the veins of the portal system. Accordingly, after trying the effects of two or three rarefactions on an animal and getting the usual results, we opened the case and ligatured the abdominal aorta above the origin of the cœliac axis. In this way nearly all the abdominal and more than half the general systemic blood-flow was blocked. If the fall of arterial pressure usually seen on extra-thoracic rarefaction were due to dilatation of cutaneous or abdominal veins, it must be much less after the aorta had been ligatured as above stated. But experiments showed that this was not the case. To take an example:

TABLE II.

Cat XXIV.	Time. H. M. S.	Normal Pressure.	Rarefaction.	Arterial Pressure.
	8 54 40	"		179 mm.
	50	"		180
	55		.8 cm.	148
	5		"	150
	10		"	154
	15		"	158
	20		"	162
	25		"	165
	8 57 40	"		166
	50	"		166
	58		1.8 cm.	94
	5		"	88
	10		"	82
	15		"	83
	4 8 40	"		204
	50	"		208
	4	"	3 cm.	76
	5		"	63
	10		"	51
	15		"	52
	20		"	52
	25		"	52

Comparison of these figures with those given in Table I shows that enlargement of the superficial and abdominal vessels can have little to do with the observed fall in blood-pressure. The effect of rarefaction is as marked after tying the aorta (which had been

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done in cat XXIV) as it was before. The question then arises, what becomes of the great mass of the blood?

S. de Jager[2] has called special attention to three main factors on which the cubic capacity of the pulmonary vessels depends. According to him, (1) expansion of the lungs by suction, as contrasted with distension due to forcing in air under pressure, causes enlargement of the pulmonary vessels in length and width. (2) The diameter of the smaller vessels varies with the intra-alveolar pressure, the two bearing an inverse ratio. (3) The capacity of the lung capillaries is influenced by the *absolute* and *relative* pressures on the exterior of the pulmonary arteries and the pulmonary veins. In connection with this statement one must bear in mind that the pulmonary arteries, with coats so much thicker than those of the pulmonary veins, do not on merely physical grounds yield to so great an extent under variations of intra-thoracic gaseous pressure.

Under the conditions maintained during our experiments the air surrounding the animal is rarefied, causing an unusually powerful inspiratory movement. Indeed, if the rarefaction passed a moderate limit, the thoracic walls during its continuance remained fixed in the inspiratory position. The increased inspiratory movements caused by the rarefaction, with their concomitant unusual distension of the lungs, must, if de Jager be correct, cause an increase in the volume of the pulmonary vessels. Intra-alveolar pneumatic pressure, de Jager's second factor, is lessened only while the lungs are in the act of distension, and even then (except to a very trivial extent) only when the distension is rapid, as it was in our experiments. After full lung expansion, a condition of pneumatic equilibrium is quickly attained in which intra-alveolar pressure is the same as before the distension commenced. While we might, therefore, expect increased capacity of the pulmonary vessels during extra-thoracic rarefaction with concomitant fall of systemic arterial pressure through the influence of the second factor, once the rarefaction was completed and kept uniform, we should have no reason to expect further change in general arterial pressure from this cause, except the normal ones due to heart-beat and breathing movements.

Pneumatic pressure on the pulmonary arteries and veins is, of course, a pressure of one atmosphere less the amount necessary to

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overcome the elasticity of the lungs; which amount depends on the extent to which the lungs are expanded in consequence of diminution of pressure on their surface. In other words, the more the lungs are distended by extra-thoracic rarefaction, the less the air pressure on the great pulmonary vessels. The pulmonary artery and vein consequently expand, with the result that the lung capillaries are filled under a lower arterial pressure and emptied against a lessened venous resistance; hence, de Jager maintains that the quantity of blood contained in the pulmonary capillaries must diminish. But when we consider the physical and mechanical differences between the coats of the pulmonary arteries and those of the pulmonary veins, it becomes clear that blood in the pulmonary artery may remain under but slight alteration of arterial pressure; while so far as such changes of extra-pulmonic pneumatic pressure can influence it, the flow in the less resisting and less elastic pulmonary veins may be considerably modified. If the lung veins expand while the lung arteries remain practically of their previous calibre, the vein expansion may compensate or even over-compensate the capillary emptying, and the volume of blood in the lungs at any moment remain the same or be but little altered; and if altered, the alteration may be in the positive or negative direction. The change in the bulk of blood contained in the lungs which could possibly be brought about by transient diminution of extra-pulmonic air pressure, must in any case be so slight as to justify us in neglecting it as a factor of any importance in the explanation of the great fall of systemic arterial pressures observed when the chest was expanded by extra-thoracic rarefaction. We have found (see Table I, Cat XXIV) that even slight diminution of the atmospheric pressure on the surface of an animal's body leads to a sudden and very great fall of pressure in the systemic arteries; this was usually followed rapidly, that is, within a few seconds, by a partial recovery of arterial pressure, succeeded very soon by return to a lower arterial pressure, but not one so low as that of the primary fall; this lowered pressure was maintained approximately uniform for a considerable time. In cases where the rarefaction was greater, the fall of blood pressure was such as to lead to rapid death; the left heart apparently received no blood to

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pump into the arteries, and the mercury in the manometer ceased to show pulse-beats and slowly fell to zero.

The marked primary fall we believe due to the combined influence of the first and second factors indicated by de Jager and stated above. The continued low pressure observed in the cases where the rarefaction was not such as to cause death must be mainly due to the first factor (that is, the general enlargement of the pulmonary vessels when the lungs are expanded by diminution of external pressure), since the second factor ceases to operate when the rarefaction has reached its limit in any given experiment. It would seem that just as most of the blood accumulates in the abdominal vessels after section of the splanchnic nerves, causing a marked fall in general arterial pressure, so when the chest cavity is dilated by extra-thoracic rarefaction most of the blood accumulates in the pulmonary small arteries, veins and capillaries, causing great passive congestion of the lungs and a great and often fatal fall of aortic blood pressure. When the extra-thoracic rarefaction is great, a very large percentage of the total blood in the body is held in the pulmonary vessels—a percentage which would be surprising did we not know that the normal lung vessels must in each minute transmit as much blood as all the systemic vessels, and that their area and capacity must, considering the size of the organs they traverse, be far greater than that of the vessels of any other organ.

We do not know whether attention has hitherto been called to the fact which our experiments demonstrate—the fact that the lungs can, under some conditions, lock up and hold so much blood as to prevent any of it reaching the heart, and so cause death by what has been well-named "internal bleeding." This possibility seems to deserve the attention of clinicians and pathologists.

An interesting by-result of our work came from the counting-out of pulse-rates on the kymograph tracings. The fact that, starting with normal arterial pressure, any increase of blood pressure in the *medulla oblongata* tended to slow the pulse, as well as the converse fact, that lowering of normal arterial pressure in the cranial cavity is followed by quicker pulse, is well established. But it has been generally assumed that such pulse-slowing or pulse-quickening results from the raising or lowering of an arterial

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pressure which started from what may be called the "base line." In other words, given a normal intra-cranial arterial pressure, increase of it will (the nerves being intact) slow the pulse; while under like conditions, lowered arterial pressure in the *medulla oblongata* will result in a quicker pulse. Our experiments serve to show that the "base line" of arterial pressure has no absolute value. With low arterial pressure, further lowering quickens the pulse; with even abnormally high arterial pressure in the *medulla oblongata* and a corresponding slow pulse, an increase of pressure makes the heart for a time beat more slowly. We have not yet had time to make a thorough study of this question, but are inclined from the results of our work so far to believe that (the nervous system being intact) no matter how low blood pressure may be, provided that it is sufficient to maintain life, a lessening of it will temporarily quicken the heart-beat; and, on the other hand, no matter how high arterial pressure may be, an increase of it will for the moment slow the pulse. There seems to be no fixed "norm" above which increased arterial pressure slows, below which decreased arterial pressure quickens, the pulse: be the existing pressure what it may, increase of it slows, diminution of it quickens. We can as yet make this assertion only provisionally: though the countings of the great majority of our tracings lead us to believe it correct, we have found many exceptions, and these have to be accounted for. Possibly the dose of anæsthetic may explain the discrepancies: it was not observed with any attention during our work; as all we cared for in that connection during our pneumatic experiments was that the animal should be unconscious, and this side-issue arose only after their completion and when the pulse-tracings were counted and compared.

Description of Plate XII.

In Plate XII is given an exact copy of the tracing obtained from cat XXVI before, during, and after the first rarefaction of the air in the chamber. The corresponding figures have already been given in Table I.

The curves are to be read from right to left.

The blood pressure tracing is shown in *AB*, the base line or line of no-pressure being the straight line *CD*.

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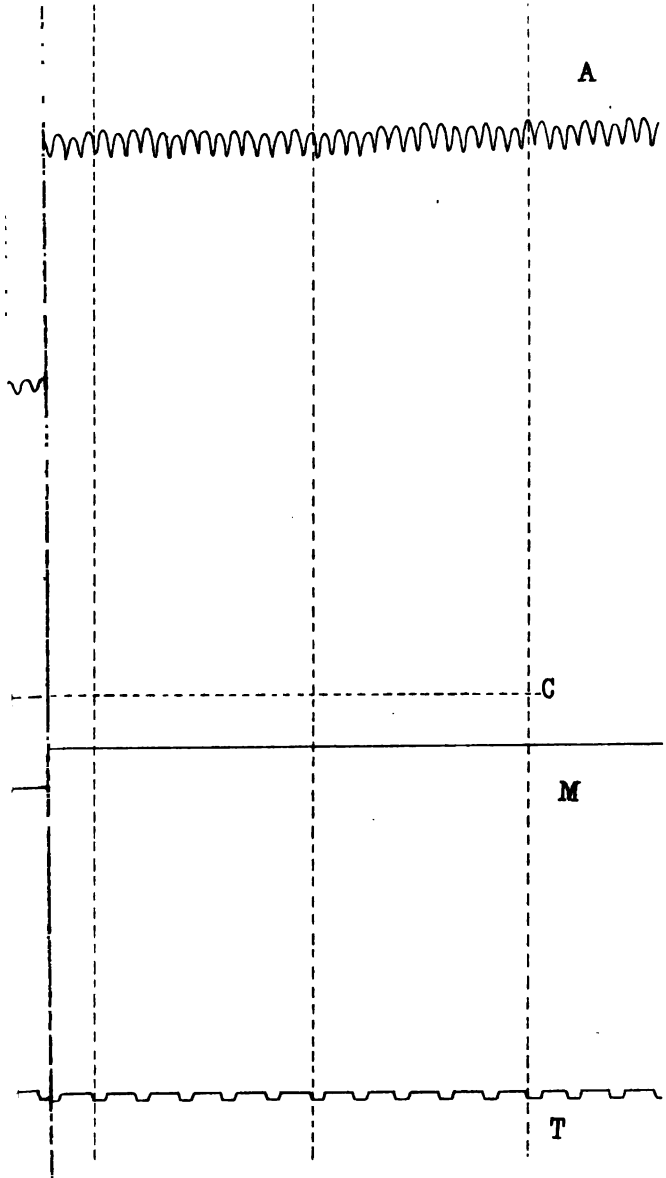

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MN shows the condition of the pressure in the case, being the normal atmospheric pressure from *M* to *O*. The rarefaction is indicated by the descent of the line at *O*, and was maintained to the point *P*, where the air was again admitted and the pressure returned to the normal.

TT' gives the tracing of a Martin pen, the time intervals marked on it being seconds.

The vertical lines were used to count the pulse rate at intervals of 5 seconds, and were retained in the proofs for convenience of verification.

The blood pressure tracing shows the initial fall of pressure at *FF'*, a temporary rise at *R*, after which it remains permanently lowered until the air enters the case as shown at *P*; at that point the blood pressure curve also goes up, as is seen at *HH'*.

References.

1. Johns Hopkins University Circulars, Vol. VI, No. 53, November, 1886.

2. Pflüger's Archiv, Vol. 36, pp. 309-346, 1885.

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SALPA IN ITS RELATION TO THE EVOLUTION OF LIFE. By W. K. BROOKS.

Salpa is distinctively a pelagic animal, adapted by its whole structure for a free existence, and for life at the expense of the micro-organisms in the water of the ocean.

To understand its position and significance in the economy of nature, we must have before us the broad outlines, at least, of a picture of the conditions under which oceanic life has been evolved.

I believe that the history of the evolution of salpa, as told by its embryology, is most suggestive and important, and that it contributes to the solution of some of the most profound and fundamental problems of biology, and brings us into conflict with some of the most favorite dicta of modern morphology. I shall therefore devote considerable space to a review of certain familiar features of ocean life, in order that I may present in this way my view of the significance of the phylogeny of salpa, in its bearing upon the first principles of morphology.

Contrast between Terrestrial Life and Marine Life.

In a picture of the land, the mind calls up a vast expanse of verdure, broken only by water, and stretching through forest and meadow from high up on the mountains, over hills and valleys and plains, down to the sea.

Our picture of the ocean is an empty waste, stretching on and on with no break in the monotony, except, at long intervals, a floating tuft of sargassum, or a flying fish, or a wandering sea-bird, and we never think of the ocean as the home of vegetable life.

It contains plant-like animals, "zoophytes," in abundance, but while they resemble plants or flowers in form and color, and in their mode of growth, they are true animals and not plants.

At Nassau, in the Bahama Islands, the visitor is taken in a small boat, with windows of plate glass set in the bottom, to visit

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the "sea-gardens" at the inner end of a channel, through which the pure water from the open sea flows between two coral islands, into the lagoon. Here the true reef corals grow in quiet water where they may be visited and examined.

The bottom of the boat is below the surface ripples and reflections. When illuminated by the vertical sun of the tropics, and by the light which is reflected back from the white bottom, the pure transparent ocean water is as clear as air, and the smallest object, forty or fifty feet down, is seen distinctly.

As the boat glides over the great mushroom-shaped coral domes which arch up from the depths, the dark grottoes between them, and the caves under their overhanging tops, are lighted up by the sun far down among the flower-animals or anthozoa and the animal plants or zoophytes which are seen through the waving thickets of brown and purple sea fans and sea feathers as they toss before the swell from the ocean.

There are miles of these "sea-gardens" in the lagoons of the Bahamas, and it has been my good fortune to spend many months studying their wonders, but no description can convey any conception of their beauty and luxuriance, and I never spent a day among the reefs without longing, at every turn, for the skill to copy with a brush the new beauties which never ceased to present themselves.

The general effect is very garden-like, and the beautiful fishes of black and golden yellow and iridescent cobalt blue hover like birds among the thickets of yellow and lilac gorgonias. The parrot fishes (Diodon and Ballistes) seem to be cropping the plants like rabbits, but more careful examination shows that they are biting off the tips of the gorgonias and branching madrepores, or hunting for the small crustacea which hide in the thicket, and that all the apparent plants are really animals. The delicate star-like flowers are the vermilion heads of boring annelids, or the scarlet tentacles of actinias, and the thicket is made up of pale lavender bushes of branching madrepores and green and yellow and olive masses of brain coral, of alcyonarians of all shades of yellow and lilac and purple and red, and of red and brown and black sponges. Even the lichens which incrust the rocks are hydroid corals, and the whole sea-garden is a dense jungle of

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animals where plant life is represented only by a few calcareous algæ, so strange in shape and texture that they are much less plant-like than the true animals.

The scarcity of vegetation becomes still more noticeable when we study the ocean as a whole.

On land, herbivorous animals are always much more abundant and prolific than the carnivora, as they must be to keep up the supply of food. Insectivorous birds are very abundant, but they are not numerous enough to keep the plant-eating molluscs and insects in check, and the devastation which is caused every year by the armies of grasshoppers and locusts and herbivorous beetles and by other less conspicuous insects, shows that their natural enemies are not numerous enough to overtax their productive power.

The birds which feed upon grain and seeds and fruit are very abundant indeed, and they sometimes gather at their breeding grounds, or places of assembly, in innumerable multitudes, but the hawks and owls which prey upon them are never numerous.

The small rodents, such as the rats, mice, squirrels, and rabbits, are the most abundant and prolific of animals; but the small carnivora are so rare that their very existence is known to few except naturalists and trappers.

The homes of the wild sheep and goats, deer, antelopes, cattle and horses support these large mammalia in incredible numbers, but their carnivorous enemies are never abundant. It is clear that if the destruction of the plant-eaters exceed their productive power, both herbivora and carnivora would disappear, and terrestrial life would come to an end.

The animal life of the ocean shows a most remarkable difference, for marine animals are almost exclusively carnivorous.

The birds which live upon the ocean, the terns, gulls, petrels, divers, cormorants, tropic birds and albatrosses, are very numerous indeed; so numerous that in many parts of the ocean some are always visible in calm weather around the vessel, wherever it may be. The only parallel to the pigeon-roosts and rookeries of the land is found in the dense clouds of sea-birds around their breeding places, but these sea-birds are all carnivorous; most of them are fishers, and others, such as the petrels, scoop up the copepods and pteropods from the surface. Even the birds of the sea-shore

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subsist almost exclusively upon animals such as molluscs, crustacea and annelids.

The seals pursue and destroy fishes; the sea-elephants and walruses live upon lamellibranchs; the whales, dolphins and porpoises, and the marine reptiles, all feed upon animals, and most of them are fierce beasts of prey. The manatee is a vegetable feeder, but it is not strictly a marine animal, since its home is in the mouths of great rivers.

There are a few fishes which pasture in the fringe of seaweed which grows in the littoral zone of the ocean, and there are some which browse among the floating tufts of algæ upon its surface, but most of them frequent these places in search of the small animals which live among the plants. All the floating fishes whose home is the floating sargassum; the file fishes and trigger fishes (Ballistida); the trunk fishes (Ostracion); the frog fishes (Antennarius); and the puffing fishes (Tetradon and Diodon) are carnivorous, living upon the barnacles and molluscs and hydroids which grow upon the sargassum, or upon the crustacea, young fishes and the floating larvæ which seek its shelter.

In the Chesapeake Bay, the sheepshead (Diplodus probatocephalus) browses among the algæ upon the submerged rocks and piles like a marine sheep, but its food is exclusively animal, and I have lain upon the edge of a wharf watching it crush the barnacles and young oysters until the juices of their bodies streamed out of the angles of its mouth and gathered a host of small fishes to snatch the fragments as they drifted away with the tide.

Many important fishes, like the cod, pasture on the bottom, but their pasturage consists of molluscs and annelids and crustacea, instead of plants.

The vast majority of marine fishes are fierce hunters, pursuing and destroying smaller fishes, and often exhibiting an insatiable love of slaughter, as in the case of our own blue-fish and the tropical albacore and barracuda. Others, such as the herring, feed upon smaller fishes and the pelagic pteropods and copepods; and others, like the shad, upon the minute organisms of the ocean, but all, with few exceptions, are carnivorous.

In the other great groups of marine animals we find some scavengers, some which feed upon micro-organisms, and others

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which hunt and destroy each other, but there is no group of marine animals which corresponds to the herbivora and rodents and plant-eating birds and insects of the land. The pelagic copepods are, of all the marine Metazoa, the ones whose place in the economy of nature is most like that of the terrestrial plant-eaters. They swarm in innumerable multitudes at the surface of the ocean, and also below it down to a depth of a mile or more, and they furnish the chief food for most young fishes, and for great armies of herrings and pteropods and jelly-fishes and siphonophores, and for most pelagic larvæ.

There are plant-eating molluscs and echinoderms and annelids in the ocean, but not in sufficient numbers to play any conspicuous part in its economy, and the copepods are the only plant-eaters which exist in sufficient numbers to be compared with those of the land, and the food of the copepods is only partially vegetable, for they devour microscopic animals as well as microscopic plants, and probably to an equal amount.

The group crustacea as a whole is a carnivorous one, however, for while a few subsist on algæ, their number is inconsiderable. Others chew the mud of the bottom and extract its organic matter, but this is chiefly animal and consists of foraminifera and rhizopods and infusoria.

The molluscs as a whole are carnivorous, and while there are many exceptions, such as the nudibranchs for example, many nudibranchs feed on hydroids.

The cephalopods and pteropods and heteropods and many of the gasteropods pursue and destroy their prey, and other gasteropods are scavengers, while the lamellibranchs gather up the microscopic organisms which are drawn into their gills with the water.

The majority of the worms and echinoderms are animal-feeders. Some of them, like the common starfish, are actively predaceous; others, like the crinoids, gather up microscopic organisms from the water; others, such as most holothurians, eat the mud of the bottom and digest out of it the foraminifera and small molluscs and annelids and crustacea which it contains, while others, such as the sea-urchins of the coral reefs, grind away and swallow the living coral. The universal presence of a poisoning apparatus in the cœlenterates shows that the food of this great and important

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group of marine animals must consist, in the main, of animals which are able to resist or to escape, and observation shows that this is true. Floating jelly-fishes and siphonophores are often found fastened to the half-digested carcasses of sagittas or heteropods or fishes larger than their captors, and they consume enormous numbers of copepods, pteropods, young fish, and pelagic larvæ of all sorts. So far as we know, all the sea-anemones and coral polyps and alcyonarians and hydroids are carnivorous. Some of the discomedusæ, the rhizostomes, feed upon microscopic organisms, but this mode of life is exceptional, and some recent observations, as yet unpublished, by Dr. R. P. Bigelow, show that the food of the rhizostomes consists of copepods.

Except for a few plant-eating fishes and molluscs and worms and echinoderms, all the animals of the ocean fall into two classes, those which subsist on microscopic organisms, and those which prey upon each other and correspond to the rapacious animals of the land.

There is practically nothing in the ocean corresponding to the terrestrial herbivora, and nothing like terrestrial vegetation, except the fringe of seaweeds in the shallow water along the coast, and a few floating islands of algæ like the Sargasso Sea.

While these tracts of vegetation are pretty extensive, they are totally inadequate to support the animal life of the ocean, and as the whole animal world is dependent directly or indirectly upon plants, we must ask what takes the place of terrestrial vegetation.

The Fauna of Mid-ocean.

There is so much room in the vast spaces of the ocean, and the part which is open to our direct observation is such an inconsiderable part of the whole, that it is only when great multitudes of pelagic animals are gathered together at the surface that the abundance of marine life becomes visible and impressive; but some faint conception of the boundless wealth of the ocean may be gained by observing the quickness with which marine animals become crowded at the surface in favorable weather.

On a cruise of more than two weeks from Cape Hatteras to the Bahama Islands I was surrounded continually, night and day, by

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a vast army of dark-brown jelly-fishes (Linerges mercutia), whose dark color made them very conspicuous in the clear water. They were not densely crowded, although they were so abundant that nearly every bucketful of water we dipped up contained some of them. We could see them at a distance from the vessel, and at noon, when the sun was overhead, we could look down into the water to a great depth through a well in the middle of the vessel where the centreboard hung, and as far down as the eye could penetrate, fifty or sixty feet at least, we could see the brown spots drifting by like motes in the sunbeam. We cruised through them for more than five hundred miles, and we tacked back and forth over a breadth of almost a hundred miles, and they were everywhere in equal abundance.

The recent literature of pelagic exploration, which has been summarized by Haeckel (Plankton Studien: von Ernst Haeckel, Jena, 1890), is full of references to great accumulations of pelagic animals, from which I have selected those which follow.

Chiercha says that during a cruise of forty days between Peru and Hawaii the net brought in from the surface and from all depths down to about two miles, a multitude of pelagic animals which would be incredible to those who have not witnessed it.

The naturalists of the Challenger found the waters of the equatorial Pacific swarming with life, not at the surface alone, but in its deeper layers, and the ship often sailed through great banks of pelagic animals.

The equatorial Atlantic is like the Pacific, and Chiercha says that its zone of equatorial calms is rich beyond all measure in animal life, and that the water often looks and feels like coagulated jelly.

Of the Indian Ocean, Haeckel says that in his voyage to and from Ceylon he was wonderstruck with the wealth of pelagic life day after day on the mirror-like surface. At night it was an unbroken sheet of sparkling light as far as the eye could reach, and the water which was dipped up at random held such a thick swarm of densely crowded luminous animals (Ostracods, Salpæ, Pyrosomas, and Medusæ) that a printed book could be read distinctly in a dark night by this pelagic light.

In temperate and arctic waters there is less diversity, but, as Haeckel shows, there is no evidence of any decrease in individuals,

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and banks of pteropods (Clio and Limacina), so dense that they seem almost solid, are met even beyond the arctic circle.

Haeckel says that in a cruise to the northwest of Scotland he met with such enormous masses of Limacina that each bucket of water which was dipped up contained thousands.

The tendency to gather in crowds is not restricted to the smaller pelagic animals, and many species of raptorial fishes are found in densely packed banks.

The fishes in a school of mackerel are as numerous as the birds in a flight of wild pigeons. Goode, in his History of Aquatic Animals, tells of one school of mackeral which was estimated to contain a million barrels, and of another which was a windrow of fish half a mile wide and at least twenty miles long; but while the pigeons are plant-eaters, the mackerel are rapacious hunters, pursuing and devouring the herrings, as well as the pteropods and pelagic crustacea.

Herring swarm like locusts, and a herring bank is almost a solid wall. In 1879 three hundred thousand river herring were landed by a single haul of the seine in Albemarle Sound; but the herrings are also carnivorous, each one consuming myriads of copepods every day. In spite of this destruction and the ravages of armies of medusæ and siphonophores and pteropods, the fertility of the copepods is so great that they are abundant in all parts of the ocean, and they are met with in numbers which exceed our powers of comprehension.

On one occasion the Challenger steamed for two days through a dense cloud formed of a single species, and they are found in all latitudes from the arctic regions to the equator, in masses which discolor the water for miles. We know, too, that they are not restricted to the surface, and that the banks of copepods are sometimes a mile thick. When we reflect that thousands would find ample room and food in a pint of water, we can form some faint conception of their universal abundance.

The Primary Food-supply.

As the result of our review, we find that the organisms which are visible without a microscope in the water of the ocean and on the sea bottom are almost universally engaged in devouring each

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other, and many of them, like the blue-fish and the albacore, are never satisfied with slaughter, but kill from mere sport.

Insatiable rapacity must end in extermination unless there is some unfailing supply, and as we find no visible supply in the water of the ocean we must seek it with a microscope. By its aid we find a wonderfully rich and diversified fauna made up of innumerable larvæ of all sorts of marine animals, together with a few minute and simple metazoa, but these things cannot form the food-supply of the ocean. It is clear that a single carnivorous animal could not exist very long by devouring its own children, and the result must be the same however great the number of individuals or species.

The total amount of these organisms is inconsiderable, however, when compared with the abundance of a few forms of protozoa and protophytes, and both observation and deduction force us to recognize that the most important element in the total amount of marine life consists of some half-a-dozen types of protozoa and unicellular plants, of globigerinæ and radiolarians, and of trichodesmium, pyrocystis, protococcus, and the coccospheres, rhabdospheres and diatomes.

Modern microscopic research has shown that these simple plants, and the globigerinæ and radiolarians which feed upon them, are so abundant and prolific that they meet all the demands made upon them and supply the food for all the animals of the ocean.

This is the fundamental conception of marine biology. The basis of all the life in the modern ocean is to be sought in the micro-organisms of the surface.

This is not all. The simplicity and abundance of the microscopic forms and their importance in the economy of nature show that the organic world has gradually shaped itself around and has been controlled by them.

They are not only the fundamental food-supply, but the primeval supply, which has determined the whole course of the evolution of marine life.

The pelagic plant-life of the ocean has retained its primitive simplicity on account of the very favorable character of its environ-

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ment, and the higher rank of the littoral vegetation and that of the land is the result of hardship.

On the land the mineral elements of plant-food are slowly supplied as the rains dissolve them; limited space brings crowding and competition for this scanty supply; growth is arrested for a great part of each year by drought or cold; the diversity of the earth's surface demands diversity of structure and habit, and the great size and complicated structure of terrestrial plants are adaptations to these conditions of hardship.

The conditions of the surface of the ocean; the abundance and uniform distribution of mineral food in solution; the area which is available for plants; the volume of sunlight and the uniformity of the temperature are all favorable to the growth of plants, and as each plant is bathed on all sides by a nutritive fluid, it is advantageous for the new plant-cells which are formed by cell multiplication to separate from each other as soon as possible in order to expose the whole of their surface to the water. Cell aggregation, the first step towards higher evolution, is therefore disadvantageous to the pelagic plants, and as the environment is so homogeneous at the surface of the ocean that there is little opportunity for an aggregation of cells to gain a compensating advantage by seizing upon a more favorable habitat, the pelagic plants have retained their primitive simplicity.

The list of pelagic micro-organisms is a long one, but a few forms are so predominant that the others have little significance at the present day in comparison, and we may regard the great primary food-supply as made up of two simple protozoa, globigerina and the radiolarians, and some five or six unicellular plants.

Of these only two, the radiolarians and the diatomes, show any great diversity of species, and while the radiolarians are so diversified that the Challenger collection alone furnished more than four thousand species, this variety does not obscure the primitive simplicity of the type, and the most distinctive peculiarity of the microscopic food-supply of the ocean is the very small number of the forms which go to make up the enormous mass of individuals.

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The Origin of Pelagic Animals.

All the animals of the ocean are dependent upon the microscopic food-supply, and many of them are adapted for preying upon it directly. Among these Salpa is one of the most conspicuous examples. It passes its whole life in the open water, and it has no sessile stage in its ontogeny, as many floating animals have. It abounds in all parts of the ocean, and over some great seas it is always present at the surface. As the result of three years' observation, Schminkewitch says that the salpas are perennial pelagic animals, and Chun has shown that they are also found in abundance at great depths.

As long as it is alive and breathing a steady stream of microorganisms is slipping along its pharynx and down through its œsophagus into its stomach, and sections of the intestine of salpa afford most beautiful preparations of radiolarians and diatomes.

The pelagic food-supply is very ancient, and we have, in salpa, an animal which has been especially evolved to pass its life swimming through the living broth of the mid-ocean.

If we were to select the typical pelagic animal we should probably choose salpa, and it is therefore most surprising to find that salpa itself has not been produced at the surface of the ocean by gradual evolution from a simple pelagic ancestor.

The structure which fits it so well for its mode of life has come to it by the inheritance of peculiarities which were originally acquired by bottom animals in adaptation to the needs of a sessile life.

This is all the more remarkable since both salpa and its fixed allies show by their embryology that still more remotely they are descended from a pelagic form like appendicularia.

The place in the pelagic world which Salpa fills so well has been ready for it from primeval times.

Why then has not the simple pelagic appendicularia given rise, in the open sea, to series of more and more perfected pelagic descendants culminating in salpa?

Why should the descendants of a pelagic ancestor have passed through a sessile stage before they acquired their improved pelagic structure?

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If this were a solitary case it would not deserve notice; but examination will show that no highly organized animal has ever been evolved at the surface, although all depend on the pelagic food-supply.

The animals which now find their home in the open waters of the ocean are, almost without exception, the descendants of forms which live upon or near the bottom or along the sea-shore or upon the land, and the exceptions are all simple animals of minute size. The metazoa which are primitively pelagic, that is, those which have been pelagic throughout their whole history and do not owe their structure to competition with improved forms from the bottom or the shore, are astonishingly few, and these few are among the smallest and simplest of the metazoa.

It is only necessary to review the chief groups of metazoa in order to perceive that most of their pelagic representatives exhibit the clearest evidence of descent from forms which lived upon or near the bottom or the shore. Many indeed have no pelagic members, but are restricted to the bottom.

The sponges are obviously a bottom group; most of them are fixed, all are sedentary, and their whole organization is an adaptation for life in the bottom.

The coral polyps, actinias and alcyonarias, are among the most characteristic bottom forms, and the abundance of the fossil remains of polyp skeletons proves that these animals became established on the bottom very early, and that the whole history of their evolution has taken place at the bottom. The acraspedote medusæ are universally and justly regarded as the descendants of fixed polyp-like ancestors, and we may state with confidence that they are not primitively pelagic, but that a fixed period in their history has come between the modern swimming jelly-fish and its remote and unknown primitive pelagic ancestor.

The veiled medusæ are usually held to have had a similar history, but I shall soon give my reasons for holding that some of these at least are primitively pelagic. There can, however, be no doubt that the evolution of hydroid cormi has taken place at the bottom. The siphonophores are descended from ancestors like the anthomedusæ, and the various families and genera and species of siphonophores have most certainly been produced by divergent special-

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ization among pelagic forms, and the greater part of their history, if not the whole of it, is therefore pelagic.

The echinoderms are most characteristic inhabitants of the bottom, as they have been from palæozoic times, and while synapta is sometimes found at the surface of the open ocean, this is exceptional, and we may state without hesitation that the evolution of the echinoderms has taken place at the bottom. This is equally true of the brachiopods and of most of the animals classed as vermes, the gephyreans, bryozoa, nemertians, and so forth. The pelagic annelids, such as Tomopteris, are secondary modifications of bottom forms, and while some of the more primitive annelids may possibly be originally pelagic, the group as a whole is as characteristic of the bottom as the echinoderms.

Many groups of crustacea have pelagic representatives, and the pelagic crustacean fauna is rich and varied, but in most cases the pelagic forms show unmistakable evidence of secondary change of habit, and all the higher crustacea have been evolved at the bottom in adaptation to a bottom life.

I shall soon give my reasons for believing that there is one important exception to this rule, however, and I shall try to show that there is good ground for holding that the copepods are primitively pelagic, and that while the greater part of the history of the crustacea is bottom history, the characteristics of the crustacean type were outlined in pelagic animals at a very early period in the history of the metazoa.

The heavy calcareous shells of the molluscs could not have been acquired at the surface, and that most characteristic molluscan organ, the lingual ribbon, is adapted for attacking more solid bodies than the delicate primitive pelagic animals. The classes and orders of molluscs must have been evolved at the bottom, and there is ample evidence that the swimming shelless gasteropods and cephalopods have, like those great pelagic groups the pteropods and heteropods, been secondarily adapted for a pelagic life.

Many of the marine fishes are strictly pelagic, and the structure and habits of fishes are in all respects so well fitted for a wandering life in the open water that the pelagic habit of fishes seems at first sight to be their most distinctive peculiarity, although a little

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examination will show that there is ample evidence that it is secondary, and not primitive.

The perfection of their adjustment to a free life in the open sea is no evidence that this life is primitive, for the highest marine animals and those whose adaptation to a pelagic life is most complete, the sea-birds and cetacea and marine reptiles, are air-breathing terrestrial animals which have gone back into the ocean.

The most primitive groups of living fishes are the cyclostomes, elasmobranchs and ganoids. The cyclostomes are too small a group, and the living forms are too aberrant in habit, to contribute much information regarding the nature of the primitive vertebrates, but they exhibit no evidence of adaptation to a pelagic life, and our scanty knowledge of them is quite in harmony with the view that their remote ancestors were bottom animals.

The case is very different as regards the great groups of modern fishes for which the term palæichthyes is often used; the sharks, rays and ganoids.

The living representatives of these great and ancient groups are of peculiar interest to naturalists on account of their close affinity to the oldest vertebrate fossils which have been discovered. These points of resemblance to the more modern, but still ancient, amphibia and teleosts show that the modern palæichthyes have preserved their ancient structure with very slight modification, and that we have in them one of the most remarkable stem forms in the whole animal kingdom. This is shown still more conclusively by the fact that some of the palæozoic families of elasmobranchs have lived through period after period of geological history and have held their ground up to our own times.

The abundance and variety of the remains of elasmobranchs in the palæozoic rocks prove the great development of the group at this remote and early period, and the silurian sharks probably differed but little from those of the present day, although we are forced to see in them the ancestors of the ganoids and of all the divergent groups of extinct and living vertebrates.

Of the three groups of modern elasmobranchs, two, the chimæras and the rays, are bottom-feeders. The whole organization of the ray is as obviously adapted for life upon or near the bottom as that of a bird is for life in the air, and the flat pavement teeth are adapted

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for crushing and grinding the hard-shelled molluscs and crustacea and echinoderms of the bottom.

It is true that the sawfish is not confined to the bottom, and the devil-fishes often capture their prey at the surface. In the West Indies they are often found very far from land, but these cases are exceptional, and the true rays rarely leave the bottom, nor are they adapted for rapid movement through the water.

The rays are undoubtedly much more modern than the true sharks, but there is ample evidence that they have retained habits of life which are common to all the primitive elasmobranchs.

Many of the modern sharks live on or near the bottom, where they are found in immense numbers and at considerable depths. In 1888 I was invited by Marshall McDonald, the Superintendent of the U. S. Fish Commission, to make use of the opportunity for surface collecting which was afforded by an expedition which was sent out to fish with hook and line on the bottom and along the edge of the Gulf Stream. The fishing commenced at the 500 fathom line, and every time the line was taken in we found numbers of dogfish (Scyllium) on the hooks, even when the water was considerably more than half a mile deep.

Many genera of sharks, such as the houndfish (Mustalus) and the dogfish (Scyllium), are known to feed upon the molluscs and crustacea and worms of the bottom, and the flat pavement-teeth of other genera whose habits are less known show that their mode of life is the same. Some of the bottom-feeding sharks (Cestracion for example) are the oldest of living vertebrates.

The mailed ganoids were undoubtedly derived from a shark-like ancestor, and the structure of the oldest ones, such as perichthys, coccosteus, and cephalaspis, shows that they were not very rapid swimmers. They were, undoubtedly, bottom-feeders like the modern sturgeon, and like many large and important families of modern teleosts, such as the cod, the siluroids and the pleuronectidæ.

So far as we know the palæozoic waters from fossils, there were no active locomotor animals of large size to furnish prey for raptorial fishes and the existence at the present day of so many species and genera and families of bottom-feeders, and the fact that the most archaic forms have this habit, are all grounds for

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believing that the fishes are secondarily adapted to a pelagic life, like the sea-birds and the cetacea.

So far as amphioxus furnishes evidence, this bears in the same direction, for its home is in the sand of the bottom. In fact it may almost be called a subterranean animal, for when it is placed in an aquarium it sinks into the sand at the bottom and disappears at once, and it makes its way through the sand with great ease and rapidity.

All the evidence shows that the primitive vertebrates lived upon or near the bottom, and that the early steps in the evolution of the classes of vertebrated animals were made at the bottom.

As the result of this review we see that the evidence from palæontology, from embryology, and from the structure and habits of living animals all bears in the same direction, and shows that there are no large or highly organized animals which have been pelagic through all the stages of their evolution, and that, in this particular, the life-history of Salpa is not exceptional, but typical.

In its descent from an inhabitant of the bottom and in its secondary adaptation to a pelagic life, its history resembles that of all the highly organized pelagic animals.

Embryology also gives us good ground for believing that Salpa follows the analogy of all the metazoa in its still more remote descent from a small and simple pelagic ancestor, and there is good ground for believing that the earliest metazoa were all pelagic, and that they were represented at a very early period in the history of life by floating or swimming animals of minute size and simple structure. We may see in the free larval forms of many marine metazoa, such as the tornaria of balanoglossus, the swimming echinoderm larva, the ascidian tadpole, the floating ciliated larvæ of annelids, brachiopods and molluscs, in the cœlenterate planula, and, as I believe, in the crustacean nauplius, traces of this primitive mode of life; often obscured or complicated by more recent adaptation and sometimes almost obliterated by secondary changes.

When this fact is seen in all its bearings and its full signficance is grasped, it is certainly one of the most noteworthy and instructive features of the history of evolution.

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The food-supply of the ocean consists of a few species of unicellular microscopic plants, and of a few simple protozoa which feed upon them. This supply is inexhaustible, and it is the only source of food for all the inhabitants of the ocean, except a few which live upon floating sargassum and the littoral algæ, and the drainage from the land.

Many marine animals are adapted for direct subsistence upon these organisms, and some of them, like Salpa, are universally distributed and are found in enormous numbers in all parts of the ocean.

The food-supply is not only inexhaustible, it is also primeval, and all the life of the ocean has gradually taken shape in direct dependence upon it during the history of its evolution.

In view of all these facts we cannot but be profoundly impressed by the thought that all the highly organized marine animals are products of the bottom, or of the shore, or of the land, and that while the largest animals on earth are pelagic, the few which are primitively pelagic are very small and very simple.

The reason is obvious. The conditions of pelagic life are so easy that there is no fierce competition, and the inorganic environment is so simple that there is little chance for diversity of habits.

The growth of terrestrial plants is limited by the scarcity of food, but there is no such limit to the growth of pelagic plants or the animals which feed upon them, and while the balance of life is undoubtedly adjusted, competition for food is never very fierce even at the present day, when the ocean swarms with highly organized animals which have become secondarily adapted for a pelagic life. Even now the destruction or escape of a microscopic pelagic organism depends upon the accidental proximity or remoteness of an enemy rather than upon defense or protection, and survival is determined by space relations rather than by a struggle for existence.

The abundance of food is shown by the ease with which wanderers from the land, like birds, find places for themselves in the ocean, and the rapidity with which they spread over its whole extent.

As a marine animal, the insect, halobates, must be very modern as compared with most pelagic forms, yet it has spread over all

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tropical and subtropical seas, and it may always be found skimming over the surface of the water as much at home as a gerris in a pond. I never found it absent in the Gulf Stream when conditions were favorable for collecting.

The easy character of pelagic life is also shown by the fact that the larvæ of innumerable animals from the bottom and the shore have retained their pelagic habit, and I shall soon refer to facts which prove that the larva of a shore animal is safer at sea than it is near the shore. The absence of fierce competition in the open ocean is well shown by the simultaneous existence in the modern ocean of graded stages in the evolution of a type, such as the series of Pelagidæ; and also by the persistency of a stem form like the elasmobranch, side by side with, and often in competition with, various improved lines of divergent descendants.

In the primitive pelagic fauna and flora there was little opportunity for an organism to gain superiority by seizing upon an advantageous site or by acquiring peculiar habits, for one place was like another, and peculiar habits could count for little in comparison with accidental space relations.

After the pelagic fauna had been enriched by the addition of all the marine animals which are secondarily pelagic, competition with these improved forms from the bottom brought about improvements in those which were strictly pelagic in their origin, and through this competition, complicated animals of considerable size, like the siphonophores, have been evolved at the surface, but while their whole history has thus been pelagic they are not primitively pelagic; that is, they are not the outcome of purely pelagic influences. The wanderers from the bottom have introduced another factor in the evolution of pelagic life, for their bodies have been utilized for purposes of protection or concealment or on account of other advantages, and we now have fishes which shelter themselves in the poisoned curtain of physalia; crustacea which live in the pharynx of salpa; barnacles and sucking fishes fastened to whales and turtles, besides a host of external and internal parasites. The primitive ocean furnished no such opportunity, and the conditions of pelagic life must, at first, have been extremely simple.

Among the higher metazoa and the higher plants size is, in itself, an important factor in evolution. Variations in the con-

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stituent cells of a large organism are continually being seized upon and fixed by natural selection, on account of their value in the functions of relation to other parts. Primitive pelagic organisms are all minute, and it is easy to understand why. To plants which are bathed on all sides by food, like the pelagic protophytes, small size is advantageous, since a small body has a larger surface in proportion to its bulk than a large one; and the pelagic plants are, as I have shown, most favorably placed for rapid growth when new cells separate as soon as they are formed, and thus expose all their surface.

The same ratio between bulk and nutritive surface tends to limit in the same way, if not to the same degree, the growth of the pelagic animals which live in the midst of an abundant supply of vegetable food.

Competition was not entirely absent among the primitive pelagic organisms, for the conditions of life are never absolutely uniform, although the possibilities of evolution must have been extremely limited and the progress of divergent modification very slow, so long as life was restricted to the waters of the ocean.

There can be no doubt that pelagic life was abundant for a long period during which the bottom was uninhabited. The history of the slow process of geological change by which the earth gradually assumed its present character, presents a boundless field for speculation, but there can be no doubt that the surface of the primeval ocean became fit for life long before the deeper waters or the sea-floor.

The early steps in the evolution of plants must have been taken in the transparent surface water under the influence of sunlight, and as both animals and plants are dependent upon oxygen, the primal flora and fauna must have lived in aerated water. The oxygen which is diffused through the ocean from the surface, where it is absorbed from the air, is gradually exhausted by oxidizable substances, both inorganic and organic, and it diminishes with the distance from the source of supply at the surface. The oceanic circulation tends to equalize its distribution, and no part of the ocean now seems to be totally without oxygen. Oxygen has been shown to be reduced to a minimum at the bottom of some of the great depressions of the sea-floor, and it is clear that a slight

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change in the conditions which influence it might render the sea-bottom unfit for life.

In early palæozoic times the sea-floor was perhaps more level than it is now, and there may have been no deep hollows like those in which the oxygen is now found to be deficient, but the average depth must have been considerably greater, when all the water which is now locked up in the sedimentary rocks of the bottom and of the shores was still free in the ocean. The circulation may also have been less active when geographical conditions were more simple, and the air was undoubtedly less rich in oxygen in early palæozoic times than it is at present.

It is therefore easy to understand that long after the crust of the earth had acquired essentially its present character, there may have been a period when the supply of oxygen was so scanty that the activities of pelagic organisms and the products of their decomposition used it up in the surface water, so that life on the bottom was impossible at a time when the superficial water supported a luxuriant fauna and flora.

During this period the proper conditions for the production of large and complicated organisms did not exist, and while the total volume of life was probably very great, it consisted of the organisms of minute size and simple structure which I have termed the primitive pelagic fauna and flora.

The Primitive Pelagic Fauna.

In using this term I do not, of course, intend to imply that these organisms are the beginning of life, or to express any opinion as to the way in which life first came into existence. I use it merely as a convenient designation for the total sum of the organisms which have been evolved by purely pelagic influences from a starting-point which is absolutely unknown at present.

The attempt to reconstruct in imagination the primitive pelagic fauna and flora is most fascinating, but all the available evidence is indirect, and as we can have little hope of finding any record of it in the rocks, we must trust to deduction rather than observation.

The modern pelagic protophytes have probably retained nearly their ancient form, but the modern radiolarians and pelagic fora-

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minifera exhibit indications of secondary adaptation, and they have undoubtedly been modified by competition with improved organisms from the bottom.

All the metazoa have pelagic larvæ, or else larval or embryonic stages, which must be regarded as the degenerated vestiges of a pelagic habit; but in most cases these larvæ have been so much changed by the accelerated development of adult features, or by the acquisition of habits or structures to fit them for the conditions of modern pelagic life, that we can deduce little more from them than the former existence of pelagic ancestors. When a pelagic larva is still represented by a modern pelagic adult of minute size and simple structure, as the tadpole larva of ascidians is represented by Appendicularia, we may be confident that it is a pelagic production, and that it existed in the primitive pelagic fauna, although this view is directly opposed to accepted dogmas regarding the origin of the Chordata. When all the members of a great group have a definite pelagic larval stage which adheres to the same plan of structure in all of them, we may be pretty confident that this larva is the representative of a primitive pelagic adult animal, even if this ancestor has now no unmodified descendants.

To my mind the best example of the retention, by all the members of a great group, of a larval stage which represents an extinct primitively pelagic ancestor is to be found in the crustacean nauplius, and notwithstanding the popular verdict against it, I do not hesitate to regard the nauplius as a pure pelagic product, and to include it in the primitive pelagic fauna, although I shall discuss this question further on. In cases like that of the echinoderms, where the pelagic larvæ of the various classes and orders are very different from each other in the details of their organization, we are hardly safe in assuming more than the primitive existence of an unknown pelagic organism, from which they have been derived. This is true to even a greater degree of the trochic larvæ of annelids, molluscs, etc., but while there is little ground for regarding the forms of these modern larvæ as ancestral, we must regard their pelagic habit as an inheritance from unknown ancestors in the primitive pelagic fauna, in which we must therefore include representatives of such larvæ as the molluscan veliger, the nemertain pilidium, the actinotrocha of phoronis, the brachiopod larva, the cœlenterate planula, and so

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forth, although we are quite unable to say how many independent starting-points these various metozoic lines had in the primitive pelagic fauna, or what these starting-points were like. Our inability to describe or picture these ancestral forms is no reason for doubting their reality, for in biology the weight and certainty of a deduction are often independent of its definiteness. We may, for example, feel sure that the cetacea are descended from terrestrial animals and yet find it impossible to picture their ancestor, or even to decide whether their ancestral lines converge into one stem before or after the pelagic habit was acquired.

We may in the same way feel sure, even in the absence of sufficient evidence to trace their direct paths, that all the great groups of metazoa ran back to minute pelagic ancestors, and we must, therefore, include in the primitive pelagic fauna a great, but indefinite, number of distinct and somewhat widely separated ancestral forms, and together with them, no doubt, an equal or greater number of somewhat similar forms which have been exterminated and have left no descendants. In these extinct forms we should, if we could study them, find the connecting links between divergent groups, and we would thus be able to complete the genealogical tree of the metazoa by bringing together the great divergent branches of the metazoic stem whose primary relationships now seem beyond discovery.

In addition to the primitive pelagic animals which are known to us only by the traces of their former existence which they have left in the structure and habits of modern larvæ and embryos, there are a few modern pelagic adult animals which show by their minute size and simple structure and by their systematic affinities that they are primitive pelagic animals, owing their structure to purely pelagic influences.

Appendicularia is a good example of this class, and I believe that the copepods are the most important group of the primitively pelagic metazoa.

The Origin of the Crustacea.

The view that the copepods are degenerated descendants from crustacea like the phyllopods, and that the crustacea were evolved on the bottom, and that the pelagic habit of the copepods is

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secondary, is so generally accepted that it is hardly worth while to advance a different view in this place where there is no room for its exhaustive treatment.

The consideration which seems to have the greatest weight with morphologists is the supposed necessity of a phylogenetic explanation of metamerism, but a little reflection will show the persistent existence of an influence which tends to metamerism at the present day.

For this influence, which is shown by such phenomena as the inheritance by the child of polydactylous feet from the polydactylous hands of the parent, or the development of wing-feathers on the toes of fantail pigeons; the influence which has carried the feet of the horse family along the same line of evolution with the hands, I have, in another place, proposed the term ontogenetic inheritance. Among the arthropods, examples of this sort of modern metamerization are very common, both as normal features of their structure, in the movable body-rings of the ocular and antennary somites of stomatopods, for example, and as monstrosities, as in the twelve-legged coleoptera.

I believe that a thorough study of this most interesting and instructive class of facts will convince any one that there is no philosophical necessity for assuming that the primitive crustacean had a highly metamerized body like that of a phyllopod, and that all the common features in the structure of arthropods may have been derived from a common ancestor as simple as a nauplius.

The analogy between the parapodia of annelids and the limbs of crustacea has been held to prove that the primitive crustacean limb was not a rowing organ fitted for a pelagic life, like the limbs of the nauplius and the copepod, but flat and leaf-like and adapted for movement over the bottom.

It is hardly possible, however, to believe that the arthropods have been derived from the higher polychætous annelids, and as the simpler and more primitive annelids have no parapodia, the resemblance, which is not actually very noteworthy, can be nothing more than an analogy.

There are plenty of degenerated copepods, and we have in their structure abundant proof of the degeneracy, and an adequate explanation of it in their parasitic habits, but they are degenerated

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descendants of ordinary swimming copepods, and not of phyllopods, and there is no reason for holding that the copepodan type itself is degenerate, except the supposed exigencies of morphological philosophy.

The active locomotor habits of the eucopepods of the open ocean would seem to be conducive to advancement rather than to degeneration, and the occurrence of phyllopods in the lower Cambrian is, of course, no more evidence that they are primitive crustacea than the occurrence of pteropods and gasteropods is that they are primitive molluscs.

I am unable to see any valid objection to the view that the copepods are primitively pelagic; that they have been evolved at the surface of the ocean from pelagic nauplii, and that the great group crustacea has been derived from them.

We have already seen that the eucopepods are the chief intermediary between the micro-organisms of the ocean and the larger and higher marine animals; that they prey upon the protophytes and protozoa, and in their turn supply either directly or indirectly most of the food for the large inhabitants of the water; that most pelagic larvæ feed upon them; that they are the food of the great pelagic banks of pteropods and heteropods, of many cœlenterates, of the young of most fishes, and of some of the most abundant and important adult fishes, like the herring, and that the sea-birds, the cetacea, and in fact almost all of the larger pelagic animals, prey upon animals which in their turn prey upon copepods.

The animals which are most important at one period in the earth's history are often replaced by others at another period, and it is, of course, possible that the modern copepods now fill a place which was in former times filled by something else; but as their organization, as compared with that of the other crustacea, exhibits all the characteristics of a primitive pelagic stem-form, and inasmuch as the remains of animals, like the pteropods, which now live almost entirely upon copepods, are found in the oldest fossiliferous rocks, there is every reason to believe that the group formed an important constituent of the primitive pelagic fauna.

No one who advocates at one time the morphological heresies which are involved in the view that appendicularia is a stem-form which is pelagic in its origin; that the nauplius is a persistent

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representative of the primitive crustacea, and that the whole history of the copepods has been pelagic; and that the veiled medusæ have been evolved in direct relation to pelagic influences; no one who makes these statements can hope to escape the charge that his view "ist die unwahrscheinlichste von Unwahrscheinlichkeiten."

The books all tell us that the free active appendicularia is the "degenerated" descendant of an ancestor which crept over the bottom; that the nauplius is a secondary larval form; that the active free-swimming copepods are degenerated phyllopods; and that the locomotor hydro-medusa is, in its origin, a specialized member of a sessile, polymorphic, hydroid cormus.

The first of these opinions, that appendicularia is a degenerated form, rests upon a supposed necessity for deriving the body of a vertebrate, which consists of a series of segments homologous with each other, but highly differentiated among themselves, from an ancestral aggregation of similar, but less differentiated, segments. The second opinion, that the copepods are degenerated and that the nauplius is a secondary larval form, is the result of a supposed necessity for explaining the segmentation of the arthropods in the same way, while the third view has its origin in the belief that the polymorphic members of a hydroid cormus must have arisen through specialization and division of labor from an ancestral undifferentiated aggregation.

These are a few, from among many, illustrations of the general acceptance among morphologists of a dogma which, while it is often refined and qualified until its character is almost lost, may be broadly stated as a belief that the homology between different parts of the same organism is always to be explained, like the homology between corresponding parts of different animals, as the result of phylogenetic inheritance; or, to state it in a different way, that the vegetative duplication of parts in animals has a phylogenetic significance, and implies descent from a duplicated ancestor.

The dogma is not the dictum of any one teacher, and it has grown almost imperceptibly from its starting-point in the discovery that the body of a metazoon is an aggregation of cells, each with an individuality of its own, specialized and differentiated by polymorphism and division of labor, and each one homologous with an unicellular organism.

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The dogma has been a most useful and suggestive working hypothesis when well controlled, but when uncontrolled it has led to the most fantastic and grotesque unscientific speculation. The climax of inconsistency into which its blind adherents have been led was well shown by the simultaneous appearance, in a recent morphological journal, of two memoirs, one an essay on "The Origin of the Vertebrates from the Arachnids," and the other on "The Origin of Vertebrates from a Crustacean-like Ancestor."

After my first examination of the second of these memoirs I laid it down, much distressed in mind by the thought that this author had unkindly descended from the sphere of experimental research in physiology, to expose the unscientific methods of the morphologists by a severe and well merited, if somewhat ponderous, satire.

In my next chapter on the morphological significance of appendicularia I shall try to show that there is no philosophical necessity for a phylogenetic explanation of duplicated structures in animals, whether they are radical, bilateral, metameric or indefinite, and I must refer the reader to that chapter for my reasons for including appendicularia, the copepods and the veiled medusæ among the primitive pelagic animals.

The Phylogeny of the Metazoa.

The primitive pelagic fauna, before the influence of the bottom and of the shore had been brought to bear upon it, consisted of small animals of simple structure; but we are forced, by the facts of comparative anatomy and embryology, to believe that a number of distinct types of structure were found among them.

Most of the great metazoic stems show by their embryology that they run back to simple and minute pelagic ancestors, and that their common meeting-point must be projected back to a still more remote time, before the differentiation of their pelagic ancestors had been effected. After we have traced each great line of metazoa as far back as we can from the study of fossils and by the aid of comparative morphology, we still find these lines distinctly laid down. The lower cambrian crustacea, for example, are as distinct from the lower cambrian echinoderms or pteropods or brachiopods

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or lamellibranchs, as they are from those of the present day. The efforts of anatomists and embryologists to reconstruct the primary phylogeny of the metazoa have so far yielded few trustworthy results, and the results which are most trustworthy are usually those which are the most indefinite.

We are therefore forced to believe that the early steps in the establishment of the various types of metazoa were taken under conditions which had some essential difference from those which have prevailed, without any fundamental changes, from the time of the oldest fossil to the present day; and we are also forced to believe that most of the great lines of descent were represented at some time in the remote past by ancestors which, living a different sort of life, differed essentially in structure as well as habits, from the representatives of the same types which are known to us. Furthermore, embryology teaches that each great group still bears internal evidence of descent from pelagic ancestors, and while the characteristics of these ancestors are in most cases unknown, a few, like appendicularia, are still found alive.

Our knowledge of the evolution of the metazoic types has certain general features which are essentially the same for all, but each group has also in its history much that is individual, and any general statement requires so much qualification that the history of an illustrative group is more instructive than a general summary.

In the echinoderms we have a well-defined type represented by abundant fossils, very rich in living forms, very diversified in its modifications, and therefore well fitted for use as an illustration.

This great stem contains many classes and orders, all constructed on the same plan, which is sharply isolated and quite unlike the plan of structure in any other group of animals. All through the series of fossiliferous rocks echinoderms are found, and the plan of structure is always the same. Palæontology gives us most valuable evidence regarding the course of evolution within the limits of a class as in the crinoids and in the echinoids; but we appeal to it in vain for light upon the organization of the primitive echinoderm, or for connecting links between the classes. To our questions on these subjects and on the relation of the echinoderms to other animals, palæontology is silent, and throws them back upon us as unsolved riddles.

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The morphologist unhesitatingly projects his imagination, held in check only by the laws of scientific thought, into the dark period before the times of the oldest fossils, and feels absolutely certain of the past existence of a stem-form, from which the classes of echinoderms have inherited the fundamental plan of their structure, and he affirms with equal confidence that the structural changes which have separated this ancient type from the classes which we know were very much more profound and extensive than all the changes which each class has undergone from the earliest palæozoic times to the present day.

He is also disposed to assume, but, as I shall show, with much less reason, that the amount of change which structure has undergone is an index to the length of time which the change has required, and that the period which is covered by the fossiliferous rocks is only an inconsiderable part of that which has been consumed in the evolution of the echinoderms.

The morphologist does not check the flight of his scientific imagination here, however, for he trusts implicitly to the embryological evidence which teaches him that, still further back in the past, all the echinoderms were represented by a minute pelagic animal which was not an echinoderm at all in any sense except the ancestral one, although it was distinguished by features which natural selection has converted, under the influence of more modern conditions, into the structure of echinoderms. He finds, in the embryology of modern echinoderms, phenomena which can bear no interpretation but this, and he unhesitatingly assumes that they are an inheritance which has been handed down from generation to generation through all the ages from the prehistoric times of zoölogy.

Other groups tell the same story with equal clearness. Who can look at a living lingula without being overwhelmed by the effort to grasp its immeasurable antiquity, and by the thought that, while it has passed through all the chances and changes of geological history, the structure which fitted it for life on the earliest palæozoic bottom is still adapted for a life in the sands of the modern sea-floor?

The everlasting hills are the type of venerable antiquity; but lingula has seen the continents grow up, and has maintained its integrity unmoved by the convulsions of nature which have given to the crust of the earth its present form.

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As measured by the time-standards of the morphologist, lingula itself is modern, for its life-history still holds, locked up within it, the record of a structure and of a habit of life which were lost in the unknown past at the time of the lower cambrian, and it tells us, vaguely but unmistakably, of a life at the surface of the primitive ocean at a time when the brachiopod stem was represented by minute and simple pelagic animals.

Broadly stated, the history of each great line of metazoa has been like that of the echinoderms or brachiopods, for while the brachiopods are certainly much more closely related to the polyzoa or the gephyreans than to the echinoderms, and while these latter are nearer to the chordata than to the brachiopods, yet each great line stands sharply by itself.

The oldest pteropod or lamellibranch or crustacean or echinoderm or vertebrate which we know from fossils exhibits its own type of structure with perfect distinctness, and later influences have done no more than to expand and diversify the type, while anatomy fails to guide us back to the point where these various lines met each other in a common source, although it forces us to believe that this common source once had an individual existence.

Embryology teaches that each line once had its own pelagic representatives, and that the early stages in the evolution of each type have passed away and left no record.

The palæontological side of the subject has recently been ably summed up by Walcott in an interesting memoir on the oldest fauna which is known to us from fossils (The Fauna of the Lower Cambrian or Olenellus Zone, by Charles Doolittle Walcott, U. S. Geological Survey, 10th Annual Report, Washington, 1890).

The fossils of the lower cambrian are not absolutely the oldest known, but it is the oldest fauna which is represented with sufficient completeness for a general view, and is, therefore, interesting to biologists.

Walcott says that no plants are known in the rocks of the lower cambrian, and that he has satisfied himself, after a study of all the reputed species of algæ, that they are not plants, but the trails of worms or molluscs.

The number of species is small, but their diversity is most noteworthy and remarkable.

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Walcott's collection of 141 American species from the lower cambrian is distributed over most of the marine groups of the animal kingdom, and, except for the absence of the remains of vertebrates, the whole province of animal life is almost as completely covered by these 141 species as it could be by a collection from the bottom of the modern ocean.

Four of the American species are sponges, two are hydrozoa, nine are actinozoa, one an echinoderm, twenty-nine are brachiopods, three are lamellibranchs, thirteen are gasteropods, fifteen are pteropods, eight are crustacea, fifty-one are trilobites, and the trails and burrows show the existence of at least six species of bottom forms, probably worms or crustacea.

The most noteworthy characteristic is the completeness with which these new species outline the whole fauna of the modern sea-floor.

Nothing brings home more vividly to the zoologist a picture of the diversity of the lower cambrian fauna and of its intimate relation to the bottom fauna of to-day than the thought that he would have found, on the old cambrian shore, about the same opportunity to study the embryology and anatomy of pteropods, gasteropods and lamellibranchs and crustacea and medusæ that he now has at a marine laboratory, and that his studies in phylogeny would have had about the same form then that they have now.

Biological evidence based on embryology and anatomy and on the habits and affinities of animals is justly regarded, by zoologists at least, as a more perfect record of the early history of life than palæontology, and we accept, without question, proofs of phylogeny which refer to a time very much more remote than the age of the oldest fossils.

We must not forget, however, that our generalizations in primitive phylogeny rest for the most part on the study of swimming or floating larvæ of minute size and simple structure, which we can have little hope of finding as fossils.

In the formations which follow the lower cambrian, species gradually become more numerous, but this is due to divergent specialization, and Walcott says that if a comparison be made between the olenellus zone (lower cambrian) and the silurian fauna, the superiority of the latter in number of species, genera and families is at once apparent.

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"If the comparison be extended to class characters, the disparity between the two is very much reduced, and it is made evident that the evolution of life between the epoch of the Olenellus fauna and the epoch of the Ordvician fauna has been, with one or two exceptions, in the direction of differentiating the class types that existed in the earlier fauna."

The ground which we have covered in our review of these various broad aspects of the animal kingdom brings us, then, to the following point of view:

There are no highly organized animals which have been pelagic through all the stages of their evolution. The metazoa, which have been pelagic through their whole history, are either small and simply organized, as compared with the higher representatives of the group to which they belong, like appendicularia, or else, like the siphonophores, they have been perfected through competition with higher types.

Marine life is older than terrestrial life, and as all marine life has shaped itself in relation to the pelagic food-supply, this itself is the only form of life which is independent, and it must therefore be the oldest. There must have been a long period in primeval times during which there was a pelagic flora and fauna, rich beyond limit in individuals, but made up of only a few small simple types. During this time the pelagic ancestors of all the great groups of metazoa were slowly evolved, as well as others which have no living descendants. So long as life was restricted to the surface, no great or rapid advancement through the influences which now modify species was possible, and we know of no other influence which might have replaced these. We are, therefore, forced to believe that the differentiation and improvement of the primitive flora and fauna was slow, and that for a vast period of time life consisted of an innumerable multitude of pelagic organisms made up of a few forms. During the time which it took to form the thick beds of older sedimentary rocks the physical conditions of the ocean gradually took their present form, and during a part, at least, of this period, the total amount of life in the ocean may have been about as great as it is now without leaving any permanent record of its existence, for no rapid advancement took place until the advantages of a life on the bottom were discovered.

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The Discovery of the Bottom, and its Effect on Evolution.

We must not think of the populating of the bottom as a physical problem, but as colonization, very much like the colonization of oceanic islands. Physical conditions for a long time made it impossible, but its initiation was the result of biological influences, and there is no reason why the starting-point should be the point where the physical obstacles were first removed. It is useless to speculate upon the character of the physical obstacles; there is reason to believe that one of them, probably a very important one, was the deficiency of oxygen in deep water.

Whatever their character may have been they were all, no doubt, of such a nature that they first disappeared in the most shallow water around the coast, but it is not probable that bottom life was first established in shallow water, or before the physical conditions had become favorable at considerable depths.

The sediment near the shore is destructive to most pelagic animals, and recent explorations have shown that a stratum of water of very great thickness is necessary for the complete development of the pelagic flora and fauna. It is a mistake to picture pelagic life as confined to a thin surface stratum. Pelagic plants probably flourish as far down as the light penetrates, and pelagic animals are abundant at very great depths. As the earliest bottom animals must have depended directly upon the floating organisms for food, it is not probable that they first established themselves in shallow water, where the food-supply is not only scanty in amount but also mixed with sediment; nor is it probably that their establishment on the bottom was delayed until the great depths had become favorable to life.

The belts around elevated areas which are far enough from shore to be free from sediment and to have above them a sufficient depth of water to permit the pelagic fauna to reach its full development, are the most favorable spots, and I shall soon show that there is palæontological evidence which indicates that they were seized upon very early in the history of bottom life. It is very probable that colony after colony was established on the bottom, and after-

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SALPA IN ITS RELATION TO THE EVOLUTION OF LIFE

By WILLIAM K. BROOKS, PH. D.

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INTRODUCTORY NOTE.

(This paper consists of two chapters, VII and VIII, printed, in advance, from my memoir on the Genus Salpa, which is now passing through the press.)

In chapter VI of my memoir on the Genus Salpa I shall give, at length, reasons which, briefly stated, are as follows, for believing that, while salpa is remotely descended from a pelagic, appendicularia-like ancestor, it is more immediately derived from a sessile form, similar in its habit of life, and essentially, in structure also to the ascidians.

In the first place, comparative anatomy forces us to believe that the atrium of salpa is identical with the perithoracic and atrial chamber of ordinary ascidians, and the facts of embryology show beyond question that this is a real homology.

Writers on the embryology of salpa and allied animals have involved the history of the atrial system in unnatural obscurity, for its origin in the salpa embryo and in the aggregated salpa is in perfect accordance with the teaching of comparative anatomy, and quite irreconcilable with any view except the one which regards these structures in salpa as strictly homologous with the median and lateral atria of ordinary ascidians.

As Leuckart pointed out long ago, the atrial aperture of salpa is much nearer the mouth when it first appears than it is later, and in this respect the ontogeny of salpa exhibits evidence of an ascidian-like stage in its ancestry. The compactness of the ganglion of salpa, as contrasted with the elongated central nervous system of primitive chordata, and its position between the two apertures of the body, are also features of resemblance to the ascidians; and while there are now no traces, at any stage of its development, of numerous stigmatic gill-slits, like those of pyrosoma, there is ample

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indirect evidence that they at one time existed in the ancestors of salpa, which were in this respect, pyrosoma-like.

The muscle bands of salpa are easily intelligible as modified oral and atrial sphincters, and they are distinctly more irregular in the young than they are in the adult. In the young aggregated Salpa cylindrica, the fourth and fifth body muscles are clearly seen to arise as branches from an atrial sphincter, and some of the body muscles arise in the same way in the aggregated Salpa pinnata.

The peculiar anatomical relations of the pharynx and atrium of ascidians are generally and justly regarded as modifications which were gradually added on to the primitive tunicate type, as adaptations to a sedentary life. If salpa has been evolved from a swimming ancestor like appendicularia through an uninterrupted series of free pelagic stages, we can give no explanation whatever of its ascidian type of structure, while this is perfectly intelligible on the view that it is a modified ascidian.

W. K. Brooks.

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wards swept away, like clouds before the wind, by geological changes, and that the bottom fauna which we know was not the first.

Colonies which started in shallow water were exposed to accidents from which those in great depths were free, and in view of our present knowledge of the permanency of the sea-floor and of the broad outlines of the continents, it is not impossible that the first fauna which settled in the deep zone around the continents may have persisted and given rise to our modern life. However this may be, we must regard this deep zone as the birthplace of the fauna which has survived; as the ancestral home of all the improved metazoa.

The effect of life upon the bottom is more interesting than the place where it began, and we have now to consider its influence in the evolution of animals.

The effect of the secondary acquisition of a sedentary life by modern animals has been fully discussed by many writers, but no one, so far as I am aware, has ever considered the effect of the first settlement of the bottom by pelagic animals, all whose competitors and enemies had previously been pelagic.

It is doubtful whether the animals which first settled on the bottom secured any more food than the floating ones, but they undoubtedly obtained it with less effort, and were able to devote their superfluous energy to growth and to multiplication, and thus to become larger and to increase in numbers faster than pelagic animals.

Their sedentary life must have been favorable to both sexual and asexual multiplication, and the tendency to multiply by budding must have been quickly rendered more active. It is sometimes stated that the capacity for budding has been acquired among the metazoa as the result of a sedentary life, but this view hardly seems to be the true one. Capacity for asexual multiplication is very old, older in all probability than sexual reproduction, and there is no reason to believe that it has ever been lost even by the highest animals, for it must be regarded as nothing more, in ultimate analysis, than discontinuous growth. The tissues of all animals have vegetative power, and external influences determine whether this shall result in continuous or discontinuous growth, and a trace of the power to multiply asexually is retained even among the embryos of mammals. It is therefore wrong to speak of the *acquisition*

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of a capacity for budding, and it is not at all improbable that the primitive pelagic metazoa multiplied by buds; although the tendency to form connected cormi, and to retain the connection between the parent and the bud until the latter was able to obtain its own food and to care for itself, was a result, and probably one of the first results, of life on the bottom.

The animals which first acquired the habit of resting upon the bottom therefore soon began to multiply faster, both sexually and asexually, than their swimming allies; and their asexual progeny remaining for a longer time attached to and nourished by the parent stock, were much more favorably placed for rapid growth. As bottom animals live on a surface, or at least a thin stratum, while swimming animals are distributed through solid space, the rapid multiplication of bottom animals must soon have led to crowding and to competition, and it soon became harder and harder for new forms from the open water to force themselves in among the old ones, and colonization soon came to an end.

The great antiquity of all the types of structure which are represented among the modern metazoa is therefore what we should expect, for after the foundation for the fauna of the bottom was laid it became, and ever afterwards remained, difficult for new forms to establish themselves.

Our knowledge of the sea-bottom is for the most part from three sources: from dredgings and other methods of exploration; from rocks which were originally laid down beyond the immediate influence of the continents, and from the patches of the bottom fauna which have been gradually brought near its surface by the growth of coral reefs; and from all these sources we find testimony to the density of the crowd of animals on favorable spots.

Deep-sea exploration can give only the most scanty and fragmentary basis for a picture of the sea-bottom, but it shows that its animal life may thrive with the dense luxuriance of tropical vegetation, and Sir William Thomson says that he once brought up at one time on a "tangle," which was fastened to a dredge, over 20,000 specimens of a single species of sea-urchin.

While cruising on the U. S. Fish Commission schooner Grampus, I was interested to find that when a ground-line with baited fish-hooks had been sunk to the bottom in nearly a mile of water, several

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of the hooks dropped into the mouths of large sea anemones, so that they were brought up uninjured, and were carried more than three hundred miles to the laboratory, where they lived for some time in an aquarium.

The number of remains of palæozoic crinoids and brachiopods and trilobites which are crowded into a slab of fine-grained limestone is most astounding, and it testifies most vividly and forcibly to the wealth of life on the old sea-floor.

No description can convey an adequate conception of the boundless luxuriance of a coral island, but nothing else affords such a vivid picture of the capacity of the sea-floor for supporting life.

The marine plants are not abundant on coral islands, and the animals depend either directly or indirectly upon the pelagic food-supply, so that in this respect their life is like that of animals in the deep sea far from land.

The abundant life is not restricted to the growing edge of the reef, and the inner lagoons are often like crowded aquaria. At Nassau, my party of eight persons found so much to study in a little reef in a lagoon, close to our laboratory, that for four months and more we found new things every day, and our explorations seldom carried us beyond this little tract of bottom. Every inch of the surface was carpeted with living animals, while others were darting about among the corals and gorgonias in all directions; but this was not all, for the solid coral was honeycombed everywhere by tubes and burrows; and, when broken to pieces with a hammer, each mass of coral gave us specimens of nearly every great group in the animal kingdom. Fishes, crustacea, annelids, molluscs, echinoderms, hydroids and sponges could be picked out of every fragment, and the abundance of life inside the solid rock was most wonderful.

The absence of pelagic life in the landlocked waters of coral islands is as impressive and noteworthy as the luxuriance of life upon and near the bottom.

On my first visit to the Bahama Islands I was sadly disappointed by the absence of pelagic animals where all the conditions seemed to be peculiarly favorable.

The deep ocean is so near that, as one cruises through the inner sounds past the openings between the islets which form the outer

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barrier, the deep-blue water of mid-ocean is seen to meet the white sand of the beach, and soundings show that the outer edge is a precipice as high as the side of Chimborazo and much steeper. Nowhere else in the world is the pure water of the deep sea found nearer land or more free from sediment, and on the days when the weather was favorable for towing outside, we found siphonophores and pteropods and pelagic molluscs, crustacea, salpæ, and all sorts of pelagic larvæ in great abundance in the open sea just outside the inlets.

Inside the barrier the water was always calm, and day after day it was as smooth as the surface of an inland lake. When I first entered one of these beautiful sounds where the calm, transparent water stretches as far as the eye can reach, and new beauties of islets and winding channels open before one, as those which are passed fade away on the horizon, I felt sure that I had at last found a place where the pelagic fauna of mid-ocean could be taken home alive and studied on shore.

The water proved to be not only as pure as air, but also as empty. At high water we sometimes captured a few pelagic animals near the inlets, but we dragged our surface-nets through the sounds day after day only to find them as clean as if they had been hung out in the wind to dry. The water in which we washed them usually remained as pure and empty as if it had been filtered, and we often returned from our towing expeditions in the sounds without even a copepod or a zoea or a pluteus.

The absence of floating larvæ is most remarkable, for the sounds swarm with bottom animals which give birth every day to millions of swimming larvæ. The mangrove swamps and the rocky shores are fairly alive with crabs carrying eggs at all stages of development, and the boat passes over great black patches of sea-urchins crowded together by thousands, and the number of animals which are engaged in laying their eggs or in hatching their young is infinite, yet we rarely captured any larvæ in the tow-net, and most of those which we did find were old and nearly through their larval life.

It is often said that the water of the coral sounds is too full of lime to be inhabited by the animals of the open ocean, but this is a mistake, for the water is perfectly fitted for supporting the most

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delicate and sensitive animals, and we had no difficulty in keeping alive, in water taken from the sounds, the surface animals which we caught outside. Even trachomedusæ and doliolums, which are extremely sensitive to impurities in the water, could be kept alive in the house very much better than in any other place where I have ever tried to keep them, and instead of being injurious, the pure water of the sounds is peculiarly favorable for use in aquaria for surface animals.

The scarcity of floating organisms can have only one explanation. They are eaten up, and competition for food is so fierce that nearly every organism which is swept in by the tide, and nearly every larva which is born in the sounds, is snatched by the tentacles around some hungry mouth.

Nothing could illustrate the fierceness of the struggle for food among the animals on a crowded sea-bottom more vividly than the emptiness of the water in coral sounds. The only larvæ which have much chance of establishing themselves for life are those which are so fortunate as to be swept out into the open ocean, where they can complete their larval life under the milder competition of the pelagic fauna, and while it is usually stated that the pelagic habit has been retained by the larvæ of bottom animals for the purpose of distributing the species, it is more probable that it has been retained on account of its comparative safety.

There can be no doubt, in view of these facts, that competition came swiftly after the establishment of the first bottom fauna, and that it soon became very rigorous and led to rapid evolution; and we must also remember that life on the bottom introduced many new opportunities for divergent modification and for the perfecting of animals.

The increase in size, which came with the economy of energy, increased the possibilities of variation, and led to the natural selection of those peculiarities which improved the efficiency of various parts of the body in their functions of relations to each other, and this has certainly been an important factor in the evolution ot complicated organisms.

The new mode of life also permitted the acquisition of protective shells, hard supporting skeletons, and other imperishable structures, and it is therefore probable that the history of evolu-

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tion in later times gives us no index as to the time which was required to evolve, from pelagic ancestors, the oldest animals which were likely to be preserved as fossils.

Life on the bottom also introduced another most important influence in evolution—competition between blood relations. In the animals which we know most intimately, divergent modification, with the extinction of connecting forms, results from the fact that the fiercest competitors of each animal are its closest allies, which, having the same habits, living upon the same food, and avoiding enemies in the same way, are constantly striving to hold exclusive possession of all the essentials to their life. When a stock gives rise to two divergent branches, each of them escapes competition with the other, so far as they differ in structure and habits, while the parent stock, competing with both at a disadvantage, is exterminated.

Among the animals which we know best, evolution leads to a branching tree-like phylogeny with the topmost twigs represented by living animals, while the rest of the tree is buried in the dead past. The connecting form between two species must, therefore, be constructed in imagination or sought in the records of the past.

Even at the present day things are somewhat different in the open ocean, and they must have been very different in the primitive ocean, for a pelagic animal has no fixed home, one locality is like another, and the competitors and enemies of each individual are determined, in great part, by accidents. We accordingly find, even now, that the evolution of pelagic animals is often linear instead of divergent, and the early steps in the series often live on side by side with the later and more evolved forms. The radiolarians and the medusæ and the siphonophores furnish many well-known illustrations of this feature of pelagic life.

No one is much surprised to find in the South Pacific or in the Indian Ocean a salpa, or a pelagic crustacean, or a surface fish which has previously been known only in the North Atlantic, and the list of species of marine animals which are found in all seas is a very long one. The fact that pelagic animals are so independent of those laws of geographical distribution which limit land animals is additional evidence of the easy character of the conditions of pelagic life. We have seen that one of the first results of life

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upon the bottom was to increase asexual multiplication and to lengthen the time during which buds remained united to and nourished by their parents. One result of this is the crowding together of individuals of the same species, and competition between relations. We have in this and in other obvious peculiarities of life on the bottom a sufficient explanation of the fact that, since the first establishment of the bottom fauna, evolution has resulted in the elaboration and divergent specialization of the types of structure which were already established, rather than in the production of new types.

Another result of the struggle for existence on the bottom was the escape of varieties from competition with their allies by flight from the crowded spots and a return to the open water above; just as in later times the cetacea and sea-birds have gone back from the land to the ocean. These emigrants, like the civilized men who invade the homes of peaceful islanders, brought with them the improvements which had come from fierce competition, and they carried everything before them and produced a great and rapid change in the character of the pelagic fauna.

The rapid intellectual improvement which has taken place among the mammalia since the middle tertiaries, and the rapid structural development which took place in animals and plants when the land fauna and flora were first established, are well known; but the fact that the discovery of the bottom initiated a much earlier, and probably much more important era of rapid development in the forms of animal life has never received the attention which it so well merits.

If the views which I have advanced are correct, the primitive bottom fauna must have had the following characteristics:

1. It was entirely animal, without plants, and it at first depended directly upon the pelagic food-supply.

2. It was established around elevated areas in water deep enough to be beyond the influence of the shore.

3. The great groups of metazoa were rapidly established from pelagic ancestors.

4. There was a rapid increase in the size of the bottom animals and hard parts were quickly acquired.

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5. The bottom fauna soon produced progressive development among pelagic animals.

6. After the establishment of the bottom fauna, elaboration and differentiation among the representatives of each primitive type soon set in and led to the extinction of the connecting forms.

There is no reason to suppose that the first animals which were adapted for preservation as fossils have been discovered, and many of the oldest fossils, like the pteropods, are most certainly the modified descendants of simpler ancestors with hard parts, but it is interesting to note that the oldest fossil fauna which is known to us is an unmistakable approximation to the primitive bottom fauna as I have outlined it.

Walcott has given the following sketch of the broad general characteristics of the lower cambrian fauna:

The lower cambrian fossils are distributed through strata which in Washington and Rensselaer counties in New York, are nearly two miles thick, and some of them, at least, were deposited in water of considerable depth. This is shown by the fineness of the sediment and by the perfect preservation of tracks and burrows in soft mud and of soft animals like jelly-fishes. These show that the sediment was laid down slowly and gently, in water so deep as to be free from disturbance, and under conditions so favorable that it contains the remains of some animals which are not found again until we reach a very much more modern period. The fossil medusæ of the lower cambrian are so perfect that their identity is unquestionable, yet it is not until the Solenhofen lithographic slate of the Jura is reached in ascending the geological scale, that medusæ are again met with; and corals and lamellibranchs are found in the lower cambrian, although as they are not found again until the silurian rocks are reached, we have no record of their existence through the long period covered by the middle and upper cambrian.

The fauna of the lower cambrian, while it undoubtedly lived in water of very considerable depth, was not oceanic but continental, and Walcott says that "one of the most important conclusions is, that the fauna of the lower Cambrian lived on the eastern and western shores of a continent that in its general configuration outlines the American continent of to-day. Strictly speaking, the

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fauna did not live upon the outer shore, facing the ocean, but on the shores of interior seas, straits, or lagoons that occupied the intervals between the several ridges that ran from the central platform east and west of the main continental land-surface of the time."

The lower cambrian fauna was rich and varied, but it was not self-supporting, for no fossil plants are found, and the primary food-supply was pelagic. Animals adapted for a rapacious life at the surface, such as the pteropods, were abundant, and they prove the existence of a rich supply of pelagic animals. All the forms are either carnivorous animals, such as medusæ, corals, crustacea and trilobites, or they are adapted, like the sponges, brachiopods and lamellibranchs, for straining minute organisms out of the water, or for gathering up those which rained down from above, and the conditions under which they lived were obviously very similar to those on the bottom at the present day.

Walcott's studies show that the earliest known fauna had the following characteristics:

1. So far as the record goes it consisted of animals alone, and these animals were dependent upon the pelagic food-supply for support.

2. While small in comparison with many modern animals, they were gigantic in size as compared with primitive pelagic animals.

3. The species were few, but they represented a very wide range of types.

4. All the types have modern representatives, and most of the modern types are represented in the lower cambrian.

5. The habitat was not the bottom of the deep ocean, but the submerged surface of a sinking continent, under water of considerable depth.

Remains of bottom animals are found in rocks below the cambrian, and Walcott believes that while the olenellan fauna adds a little more to our knowledge of the rate of convergence backwards in geological time of the lines representing the evolution of animal life, it also proves, at the same time, that an immense interval has elapsed between the beginning of life and the epoch represented by the olenellan fauna. He says: "That the life in the pre-olenellus seas was large and varied, there can be little, it

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any, doubt. The few traces known of it prove little of its character, but they prove that life existed in a period far preceding lower Cambrian times, and they foster the hope that it is only a question of search and favorable conditions to discover it."

No one can question the validity of the basis for Walcott's hope, for pelagic animals have undoubtedly established themselves on the shores of elevated tracts again and again, during the oscillations of the sea-bottom, and we have every reason to expect and look for their remains.

If, however, it is true that the primitive stem-forms were pelagic and minute, there is little hope of finding their delicate microscopic remains in the sedimentary rocks of the shore.

The cambrian fauna is usually regarded as a half-way station in a series of organisms which reaches back into the past for an immeasurable period, and it is even stated that the history of life before the cambrian is longer, by many fold, than its history since.

So far as this opinion rests on the diversity of types in cambrian and silurian times it has no good basis, for if the view which I have advocated is correct, the evolution of the ancestral stem-forms took place at the surface, and all the necessary conditions for the rapid production of types were present when the bottom fauna first became established.

As we pass backwards towards the lower cambrian we find closer and closer agreement with the biological conception of the primitive life at the bottom.

We cannot regard the olenellan fauna as the first bottom fauna, for it contains forms which have been secondarily adapted for a pelagic life, such as the pteropods.

We may, however, feel confident that the first bottom fauna resembled that of the lower cambrian in its physical conditions, and in its most distinctive peculiarity, the abundance of types and the slight amount of differentiation among the representatives of these types.

Far from seeing in the lower cambrian fauna a half-way station in a long series of bottom animals, the biologist must regard it as an unmistakable and decided approximation to the primitive fauna of the bottom, beyond which life was represented only by simple and minute pelagic organisms.

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The Origin of the Chordata, considered in its relation to Pelagic Influences.

Section 1.—*The Ancestral Chordata.*

I shall now attempt to study the origin and significance of the structure of appendicularia in accordance with those conditions which must, as Dohrn has pointed out (Studien, etc., VIII, p. 79), direct all inquiry into the genealogy of animals.

All biologists will agree with Dohrn that no amount of morphological information, or of exhaustive microscopical study of the structure and development of animals, can suffice, in the absence of comprehensive knowledge of their mode of life and of the conditions of their existence, for the institution of inquiries into their phylogenetic relationship.

Unquestionably the first condition for genealogical inquiry is, as Dohrn says, the establishment of a direct connection between our morphological studies and the facts of physiology and biology.

"The homologies which are established by comparative anatomy, and the primitive identities which are established by comparative embryology, are only the means for this end. They are in themselves valuable in phylogenetic inquiry only so far as they furnish us the opportunity to pass from the consideration of the structure of organs as they now exist, and of the functions of these organs at the present time, to the consideration of conditions which have passed away; to the study of the history of the modifications which have come between these structures and functions and those which we must attribute to the same organs at an earlier genealogical stage."

Keeping these conditions of genealogical inquiry in view, let us try to study the structure of appendicularia in relation to the conditions of its life, so far as these are known to us, and let us see what functions we must, according to the principle of change of function, attribute to the organs of the remote ancestors of the tunicates, and what are the paths these organs have traversed in reaching their modern structure.

If the reader of the following pages should think that I wander too far from the beaten paths of observation, I must plead as my

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excuse that the study of phylogeny is impossible without the use of the imagination, and that the field is already occupied by a phylogeny of the tunicata which cannot be set aside until a more satisfactory one has been found.

Appendicularia is a very simple organism, and while much ingenuity has been expended in the negative task of accounting for the absence of all the structures which it lacks, I hope that the more positive attempt to account for its actual structure will not lead us into any great difficulties.

In the belief that the sequel will justify the assumption, I shall, as my starting-point, picture the ancestor of appendicularia as a simple, minute, unsegmented, chordate animal, leading a free, locomotor, pelagic life and subsisting upon the micro-organisms of the ocean. I shall also assume that this ancestor had an elongated, unsegmented body stiffened by an axial, unpaired, unsegmented notochord, like that of amphioxus, appendicularia, and the ascidian larva; that it had a simple, elongated, dorsal, nervous system, and an elongated, ventral, digestive tube, without pharyngeal clefts; that this tube was nearly straight; that it had a capacious lumen, and that, as in amphioxus and the tunicates, this was permanently distended and ciliated, and that the water, with the micro-organisms that float in it, was swept through it by endodermal cilia and not by muscular contractions.

In order to entangle the floating particles of food and to hold them while the water swept on through the intestine and out of the anus, gland-cells for the excretion of slime were scattered among the ordinary ciliated endoderm cells of the digestive tract. In origin, these slime-cells may have been modified or specialized digestive gland-cells.

As particles which are entangled and held captive near the oral end of the gut are more perfectly exposed to its digestive action than those which continue to float with the stream, the most anterior slime-cells are most efficient and valuable, and as each variation in this direction gave its possessor an advantage, the slime-cells gradually, through the action of natural selection, became localized in the pharyngeal region, and this region gradually became enlarged and was thus set apart, at a very early period, as a specialized tract of the gut.

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It is also probable that, at a very early stage in the phylogeny of these primitive chordata, a blind pouch was developed, behind the pharynx, to catch the food-particles as they were hurried past with the stream of water and to retain them long enough for perfect digestion, and that the rudiment of the organ which has in the higher vertebrates become the liver was thus established.

In these primitive animals the current of water through the digestive organs was most useful as the vehicle for floating food, but while necessary, it was a necessary evil, for the large distended lumen which furnished it a channel also permitted undigested food to be swept away and lost.

The immovable, permanently distended, ciliated digestive tract of a modern lamellibranch is very similar to that of these primitive chordata, but the lamellibranchs have acquired an apparatus for straining off the water from the captured food, so that the digestive tract is relieved from this disadvantageous current.

If, after the pharynx had been established, a secondary opening from it to the exterior were to be formed, this opening would permit the water to escape without passing through the intestine, and as the advantage of this new arrangement is obvious, there can be no doubt that after an opening of this sort was once formed, it would be preserved and perfected by natural selection, as a channel for the escape of the water after the food has been strained out and entangled by the excretion of the pharyngeal slime-glands.

I shall show, further on, that if an useful opening of this sort were to be fixed and preserved by natural selection on one side of the body, the laws of growth would soon cause it to be duplicated on the other side. These two openings are the so-called gill-slits of appendicularia, although they are beyond question much older than the modern appendicularia, dating back to a time before this animal had acquired the features which distinguish it from its more primitive chordata ancestors.

I am not able to suggest what led to the first establishment of a secondary opening into the pharynx; but, once formed, its preservation and gradual improvement, by natural selection, as a channel for the escape of superfluous water, and its duplication on opposite sides of the body, are easily intelligible.

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If we accept the view that the chordata type was evolved under purely pelagic influences, we are forced to believe that the first chordata were minute, and that their small bodies were soft, and unprotected by a hard covering. If we also admit that their digestive tract was a channel for a current of water, we can hardly believe that they needed respiratory organs, or, for that matter, excretory organs, for all the tissues of a minute soft animal, bathed within and without by pure water, must have been sufficiently aerated and purified without any organs for this purpose.

It is not at all probable, then, that the pharyngeal clefts were originally either gills or renal organs, and we have seen that the conditions of pelagic life furnish a much more simple explanation of their advantage, and I believe that the view that they were originally concerned in nutrition rather than in respiration will commend itself to all who approach the subject without any philosophical preconception.

After they were once established they gradually effected a rearrangement of the slime-cells and ciliated cells of the pharynx, for as it now became important that all the food particles should be entangled by the product of the slime-cells before it reached the pharyngeal clefts, the slime-cells were gradually restricted to the anterior part of the pharynx, while the ciliated cells gradually became specialized to carry the entangled food past the openings and to convey it safely into the œsophagus.

All the parts of the pharynx of appendicularia are beautifully constructed for this purpose. The pharyngeal clefts are situated far back in the pharynx, and are separated by nearly its whole width from the œsophagus. They are fringed by large cilia to expel the water, and they are separated from each other by a vertical shelf or velum on the ventral floor of the pharynx, so placed as to prevent cross-currents.

In front of this shelf the slime-cells are brought together in two rows, near the middle line, just inside the mouth, to form the hypopharyngeal band or endostyle. Between these two rows of slime-cells there is a median row of large ciliated cells, so placed that they drive the slime forwards to the point where a ciliated peripharyngeal band receives it and carries it up each side of the pharynx just behind the mouth, into the most favorable place for entangling the food, as this enters with the current of fresh water.

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On the dorsal middle line the threads of slime are gathered up and guided along the epipharyngeal band or dorsal lamella, beyond the influence of the current of water which sets backwards, on each side of the ventral velum, to the pharyngeal clefts, and the food is thus safely conducted into the œsophagus while the water escapes.

Up to this point I believe that the ancestral history of the tunicates was identical with that of the vertebrates, for the hepatic cæcum, the dilated pharynx, the pharyngeal clefts, the hypopharyngeal gland and the peripharyngeal bands have been inherited by all the chordata, and have impressed themselves so firmly in their organization that even the highest vertebrates still retain them, either as vestiges, or as organs which have been fitted to new functions.

I believe, however, that while they were acquired before the tunicates diverged from the chordata stem, they were acquired by an organism whose environment and habits of life were essentially like those of the modern appendicularia.

All the parts of the pharynx of appendicularia are so beautifully co-ordinated for effecting a purpose so useful and so well adapted to the conditions of its simple pelagic life, that we find it difficult to resist the belief that its ancestors had essentially the same habits, and that they lived under essentially the same conditions, and that this simple organization was directly acquired in adaptation to these conditions.

If this view involved any great or unusual difficulties we might well distrust it, notwithstanding its simplicity; but I shall try to show that it does not. In the preceding chapter I have shown that it accords with our knowledge of the fundamental principles of the general biology of the ocean, and further on I shall try to show that it is equally in accord with the principles of morphology.

At present we must devote our attention to the history of the evolution of the tunicates from this primitive chordata stem.

Section 2.—The Origin of the Tunicates.

Like most recent students of the tunicates, I believe that we have in appendicularia a persistent representative of the primitive tunicata; but, unlike many of them, I fail to find in its structure

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any evidence of degeneracy, or in its habits any basis for the assumption that it is degenerated. In most respects its structure is like that of the hypothetical ancestor whose evolution we have traced. It has an unsegmented notochord, and a capacious lumen throughout the whole course of the digestive tract from mouth to anus. This lumen is permanently distended and food is carried through it by cilia. It has a blind diverticulum from the stomach, and the greatly expanded pharynx opens laterally through two ciliated pharyngeal clefts, through which the water escapes while the food passes into the œsophagus. There is a ventral slime-gland just inside the mouth, and its excretion is conveyed upwards around the pharynx by the cilia of the peripharyngeal bands, and is then swept into the œsophagus with the entangled food.

This increasing complexity and perfection of the pharynx is accompanied by an increase in its size, so that in the primitive tunicates it soon comes to be the most important and dominant organ of the body, and brings about adaptive changes in other parts. One of these is the differentiation of a stomach for the retention and digestion of the food, in the direct course of the gut. As long as the food was mixed with great quantities of water, digestion and assimilation probably went on simultaneously in all parts of the post-pharyngeal gut, but as the water found another exit and the food thus became more compact and solid, the stomach of appendicularia became established and thus divided the gut into an œsophageal, a gastric, and an intestinal region.

Our knowledge of the primitive vertebrates seems to me to be too scanty to show whether this differentation occurred before or after the tunicates diverged from the ancestors of the vertebrates. We are now concerned with the history of the tunicata line alone, and the fact that the differentiation now exists in all tunicates shows that it was brought about very early in their history.

Another most important change in the relations of the gut also took place very early in their history. The intestinal portion became bent upon the enlarged pharynx so as to form a ⊏ with the intestinal bar of the ⊏ ventral to the pharyngeal portion, and with the anus on the ventral middle line under the pharynx. Herdman represents the primitive condition of the digestive tract of tunicates as a ⊏, with the intestine and anus dorsal instead of ventral (page

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128); but I shall show further on that the relations exhibited by appendicularia are the primitive ones, from which we must derive those which are exhibited by other tunicates.

By this change the tail was freed from the gut and was made much more efficient as an organ of locomotion, while the fæces were discharged from the anus into the current of water which set out through the pharyngeal clefts. This latter feature may not have been of any value so long as habits of active locomotion were retained, but, as we shall see, it became very important at a later stage.

The embryology of the ascidians shows that this arrangement of the digestive tract was secondary; that at one time it was straight, extending into that region of the body which is now specialized in appendicularia as a tail. The advantage to an active pelagic animal of this change is obvious, since it permits the tail to become purely locomotor. As each slight variation in this direction must have given a slight increase in the freedom of movement, the shape of the body of appendicularia is easily intelligible as the result of natural selection, and while the change is complete in this, the most primitive tunicate which we know, so that we can only conjecture the transitional stages, the change itself is not a complicated one. It presents little difficulty, although the resulting differentiation of appendicularia into two regions or "segments," a body and a tail, has been made the basis of much speculation.

The great development of the pharynx and the reduction of the tail to an organ of locomotion soon resulted in a pronounced change, of the sort for which Dana long ago proposed the term *cephalization*.

As the functions of the pharynx, and of its oral end in particular, became more and more complicated and more and more exactly co-ordinated, while those of the tail became simplified, the elongated nervous system became differentiated in a corresponding way, and its caudal portion became reduced to a caudal nerve, while its oral extremity became evolved into a cerebral vesicle with sense-organs and nerves in relation with the co-ordinated structures of the pharynx.

All the characteristics of appendicularia, except the structure of the heart and the structure and position of the reproductive organ, are thus seen to be intelligible as direct adaptations to a pelagic life; for its distinctive features, as compared with other primitive

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chordata, are the U-shaped folds of the digestive cavity, the sharp separation of the tail from the body, and the differentiation of the nervous system into a caudal nerve and anterior vesicle.

We have little basis for speculation as to the path by which the reproductive organ acquired its present position, and it is by no means certain whether the tunicate heart is homologous with that of the other chordata.

The conditions of pelagic life are so permanent that we may safely make use of the structure and habits of the modern pelagic forms to reconstruct this part of the ancestral history of the tunicates, for time writes no wrinkles on the azure brow of the ocean.

As regards the later history the case is different. Between appendicularia and the ascidians there is a great gap which we can bridge only in imagination. The transitional animals are totally unknown, and the conditions of life on the bottom of the modern ocean may, possibly, be very different from those which prevailed when the fixed ascidians were first evolved.

It is easy to imagine changes which might have gradually converted an ancestor like appendicularia into a descendant like the fixed ascidians, through successive adaptations to a sedentary life, but in the absence of all evidence we cannot feel implicit confidence that the imaginary picture bears any minute and detailed resemblance to the actual history.

It seems probable that after the bottom of the ocean became fit for life, some of the descendants of the primitive pelagic tunicates gradually acquired the habit of sometimes swimming upon or near it in an inclined position with the mouth downwards to suck up the organic sediment, and that they also acquired the habit of resting upon the bottom in this position.

We may well doubt whether these animals obtained any more food than their pelagic ancestors, but it is well known that it is not the amount of food, but the ratio between the supply and the amount of expended energy which affects size. As this new habit economized energy both during rest and during activity, it permitted an increase in size, and it is interesting in this connection to note that Chun has found at great depths appendicularias which may well be called gigantic as compared with all which are known to exist at the surface.

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With each increase in size, the habit of visiting the bottom must itself have become more and more fixed, until the life upon the bottom, which may have been at first only intermittent and more or less accidental, at last became established in the ancestors of the ascidians as a constant characteristic peculiarity.

As this new mode of life was gradually acquired, some method of aerating the fluids of the body must also have been gradually evolved; for without it, a minute animal adapted for a free active life in the highly aerated surface-water, could not, at the same time that it grew larger, acquire a less active habit of life in the bottom strata where the water is less perfectly aerated, the products of decomposition of organisms more concentrated, and the capacity for passing from exhausted and impure water to a fresh environment, restricted both by the more stationary habit and by the fact that life in space has been exchanged for a home which is limited by a surface.

Undoubtedly the change of habit was accompanied by the gradual perfection of the system of blood-spaces around the pharynx, which, at first indefinite and irregular, became constant on the margins of the pharyngeal clefts, which thus gradually acquired a new function and became gill-slits, and also became duplicated as the animals grew larger and the need for more perfect respiration increased with their change of habits.

I hope that no one will interpret the last sentence as an expression of the belief that the need for respiration caused the gill-slits to multiply. I believe, and shall try to show further on, that the tendency to duplicate a structure, either radially, bilaterally or serially, is a result of the method of growth by cell multiplication, and that in the case in question the serial reduplication has been fixed and preserved by natural selection on account of its value in respiration.

The context shows that I also regard the gill-slits of vertebrates and those of tunicates as homologous structures inherited from a common source, the primary pharyngeal clefts; but that I regard the increase in their number as a secondary change which has occurred in both lines after their genealogical paths had diverged.

It does not seem necessary to defend the thesis that the number of gill-slits in the ascidians is the result of secondary multiplication,

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since, as I shall show further on, it is accepted by Dohrn (Studien, etc., IX, 417), who has proved himself a most rigorous critic of the logic of morphology.

There is reason to believe that the multiplication of gill-slits in the tunicates has not only taken place independently, but that it has taken place in a peculiar way. Anatomy and embryology give evidence that while the perforations of the tunicate pharynx multiplied, the perforations of the outer wall of the body did not; and that the external portions of the two primary clefts became distended into a pair of spacious perithoracic chambers, each with numerous ciliated openings into the pharynx, and a single opening to the exterior which perhaps became enlarged as the gill-slits multiplied.

So long as the primary function of the first pair of pharyngeal clefts, the discharge of the superfluous water, was the only one, they probably remained circular like those of appendicularia; but as they became concerned in respiration and increased in number, and were furnished with definite blood-vessels, they became elongated vertically and, forming a series side by side over a considerable area on each side of the pharynx, they thus became much more efficient organs for the aeration of the blood.

In this simple way metamerism, that fetish of the morphologists, was established among the tunicates, and there is no evidence that it has ever involved any of their organs except the gill-slits and the pharyngeal blood-vessels.

A vertical series of slits, elongated longitudinally, would undoubtedly have permitted the water to escape just as well as a longitudinal series elongated vertically, but it is possible that, during the gradual establishment of the respiratory circulation, those of the irregular and variable blood-spaces which were most nearly transverse to the current of water from the mouth to the primary clefts, were the ones which were first made definite by natural selection, and that the arrangement of the gill-slits was thus determined.

We can only conjecture how this unknown ancestral swimming organism first became fixed, but the discovery of its descendants on the modern sea-floor is among the possibilities of future explanation.

The sedentary habit undoubtedly came gradually, and at first it may have been temporary, confined perhaps to the breeding season,

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when, loaded down with eggs, the animal may have learned to rest upon the fragments of crinoids, or the shells of trilobites or brachiopods or molluscs, to avoid clogging its delicate ciliated and vascular pharynx with sediment. At the point where the heavy anterior end of the tadpole-shaped body rested, the ectoderm cells, just below the mouth, probably became modified for the excretion of an adhesive cement.

The sedentary habit, which must have resulted in a still greater economy of energy and a corresponding increase of size, undoubtedly became more and more firmly established, and the changes which followed and resulted in the evolution of the ascidian type are easily intelligible as adaptations to a fixed home, although we have little to show the sequence of their acquisition.

So long as the animal led a free life the fate of the deoxidized water after it left the gill-slits had no meaning, but with the fixed habit came the need for driving it away as far as possible, and the external apertures of the perithoracic chambers became small, moved towards each other, and finally united to give to the exhaled current the strength of concentration. The attitude of the animal upon the bottom undoubtedly determined the dorsal instead of ventral location of the common aperture and of the median atrium or cloaca. As each step in this process of concentration must have been advantageous, its evolution by natural selection is easily intelligible. The accumulation of fæces from the intestine, around a fixed animal, is so unsanitary that the anus has disappeared in many sedentary metazoa, while in others, such as the crinoids and the lamellibranchs for example, secondary adaptations for sweeping away the refuse matter have been acquired.

The folding of the originally straight digestive tract of the primitive chordata into a U with the anus and intestine ventral to the pharnyx, took place in the ancestral tunicates as an adaptation to locomotion, but, as appendicularia shows, it incidentally brought the anus into the region of the pharyngeal clefts. As the sedentary habit became slowly established the anus became shifted from the middle line into the exhaled current from the left perithoracic chamber, and finally into the margin of its aperture, so that, during the migration of the exhalent openings, the U of the digestive tract

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became twisted into an 8 in such a way that, as Plate VIII, Fig. 2 shows, the intestine *p* passed on the left side of the œsophagus, *q*, to open dorsally into the atrium, near the middle line, but a little to the left.

This arrangement of the digestive organs is very characteristic of the tunicates, and the few exceptions are clearly due to later changes. Thus in doliolum the atrium has moved backwards as an adaptation to locomotion, and the anus has followed it until the gut has become nearly straight. The intestine and anus of the adult aggregated Salpa pinnata, Plate I, Fig. 1, are ventral; but I have shown that in the young the intestine crosses to the left of the œsophagus to open dorsally, as it does in the adults of all ordinary salpæ. In the Polyclinidæ the loop of the intestine has been elongated, with the elongation of the body, until the bend of the 8 has been obliterated, and the presence of the characteristic 8 in more primitive ascidians such as clavelina shows that the Polyclinidæ have been more recently modified.

All sedentary animals which take their food by means of cilia have their apertures raised in some way above the reach of sediment. In the crinoids this end is reached by a stalk; in the lamellibranchs it is attained either by siphons, or by the vertical elongation of the shell as in the oyster; and the shifting of the area of attachment of the ascidians from the oral end to the aboral end, the elongation and approximation of the mouth and the atrial aperture, the acquisition of oral and atrial sphincter muscles, the degeneration and disappearance of the locomotor tail, and the simplification of the nervous system, are such obvious adaptations to a sedentary life that it is not necessary to discuss them.

Section 3.—*The Annelidian Hypothesis.*

I believe that the structure of the tunicates has been acquired as an adaptation to the biological conditions which prevailed at the surface of the primitive ocean, and that it has been evolved by the gradual addition of successive complications on to the body of a still more primitive and simple ancestor. This involves the total rejection of the dogma that the vertebrates are modified annelids, and that the tunicates are degenerated vertebrates.

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While it is not my purpose to discuss the ancestral history of the vertebrates, the remote phylogeny of the tunicates is unquestionably identical with that of the other chordata, and I cannot ignore the general acceptance of an opinion which is absolutely irreconcilable with the one which I have presented.

This prevailing opinion has interwoven itself with the literature in such a complicated way that one may well shrink from the interminable labor which the critical revision of the whole of it would involve. I myself decline to undertake what I regard as an unprofitable and useless task; unprofitable, as the literature rests on an untenable and false basis, and useless, since I do not hope to induce those who have stored their minds with the endless details of morphology docketed and pigeon-holed according to a false system, to unload all this rubbish and to build again on a new foundation.

I shall therefore restrict myself to a discussion of the origin of the two most characteristic systems of tunicata organs, the gill-slits, and the pharyngeal ciliated cells and gland cells; and I shall here confine myself to the observations and reflections of a single writer, Dr. Dohrn.

I make this selection the more willingly, as Dohrn's name is most intimately associated with the annelidian hypothesis, and because his writings are not only the ones which have been most influential, but also the ones which are most comprehensive and most attractive to the reader.

The "Ursprung der Wirbelthiere" is a most fascinating book. Soon after it appeared I placed it in the list of works which my students are advised to read, and for many years an acquaintance with it has been expected of all who have been examined for the degree of Ph. D. in the Johns Hopkins University.

My students have even prepared for their own use an English translation of it, and I have read it with them several times with interest and pleasure. At the first reading my pleasure was almost that of conviction, but as the ingenious details became familiar, and the essay was more sharply focused in its completeness, and was held, as it were, at arm's length, so that the whole picture could be seen at one view, I have read it, as I have read Gulliver's Travels, with admiration for the skill which has elaborated it in

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such logical minuteness from a fundamental assumption which is purely imaginary.

The story, as told by Dohrn in the "Ursprung," is so consistent and logical that I see no reason why animals like the tunicates might not have been evolved in the way which he pictures so vividly, although I believe that the actual tunicates have been produced in a very different way.

I shall therefore examine the account of the origin of the gill-slits which Dohrn gives in the "Ursprung," and the view of the ciliated and glandular structures of the pharynx which is developed in his "Studien," especially in Parts VII, VIII and IX, in order to determine how far the origin of these structures is accounted for by the annelidian hypothesis, and what superiority, if any, this has over the much simpler hypothesis which is here advanced.

Dohrn says (Ursprung, p. 10) that the branchial apparatus of the tunicates and that of balanoglossus *are so much more complicated* than that of the selachians, and their origin is so much more difficult to understand, that they are of no help to us in our attempt to trace the origin of gill-slits.

I am quite at a loss for the meaning of this passage, for no secondary perforation of the pharynx could possibly be less complicated than the gill-slits of appendicularia, nor could it be developed in a simpler way than by the involution of a pit on the side of the body.

It is quite true that we do not know how the gill-slits of appendicularia first came into existence, or what influence led to their formation, but their usefulness as channels for the escape of the water which, before they were formed, must have passed through the intestine, is clear, and we can understand why they have been preserved, by natural selection, on account of this advantage.

We are forced to believe that the pharynx did, in some way, acquire a secondary communication with the exterior, although we are not able to say how it was acquired.

Dohrn's view of the origin of gill-slits is based upon the need for an explanation of the original formation of the perforation. He says (Ursprung, p. 10): "What is a gill-slit? Perforations of the body-wall do not take place directly, and still less do they form connections with corresponding perforations of the digestive tract,"

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and he therefore undertakes to study the origin and primitive function of gill-slits by the aid of the law of the change of function, and to find in a more primitive function an explanation of their present function as channels for water.

As his point of departure is the need for an explanation of the origin of the perforation, we feel a natural hope that we are to be led to this explanation, but this hope ends in disappointment.

He regards the gill-slits as modified segmental organs, but he tells us explicitly, on page 10, that "we are not able to assign any reason why segmental organs should unite with the gut," and his explanation of the origin of the perforations is no explanation at all, since it simply assumes, but does not account for, the very phenomenon which it is supposed to make clear.

His inability to understand the direct origin of the secondary perforations of the gut has one most remarkable result, for the view that the gill-slits are segmental organs involves the view (Ursprung, p. 57) that the anus of the tunicates is not a primary anus nor a secondary one, but a tertiary one, and that the ancestors of the tunicates have not only acquired two new secondary anal apertures, but that they have lost one mouth and acquired a second, and that they have lost this and acquired a third. As these mouths are supposed to be modified segmental organs, we are, according to the acknowledgment on page 10, "unable to assign any reason why they should have united with the gut."

The original mouth of the ancestors of the chordata was, according to Dohrn (page 3), on what is now the dorsal surface, and the primitive œsophagus passed through what is now known as the fossa rhomboidea of the brain.

This ancestral mouth degenerated and disappeared as it was gradually superseded in the remote progenitors of the vertebrates by a second mouth (page 5), which is the mouth of the vertebrates of the present day, and of the ancestors of the tunicates (page 57) as well, although it was gradually converted first into a sucker, and finally into an organ for fastening the tunicata to foreign bodies, while these animals gradually acquired a tertiary mouth (page 58) by the formation of a secondary communication between the nasal chamber and the gut.

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Dohrn says (page 60) that these assumptions "set the relation between the fishes and the ascidians in the right light," although the perforation of the gut, which the hypothesis is to explain, is not only left unaccounted for, but is multiplied so many times that, like the man with an unclean spirit, its last state is worse than the first.

Dohrn says that the secondary nature of the mouth of the vertebrates is proved by its very late appearance in the young vertebrate after its embryonic body and its great systems of organs are fully formed, and by the fact that, when it does make its appearance, it does not lie at the anterior end of the body, in the place which it finally occupies in the great majority of vertebrates, but at a spot some distance behind this place.

It is not possible to attach much weight to either of these arguments, for slight changes in the position of organs are not unusual, and it is well known that the ontogenetic acceleration or retardation in the relative time of appearance of structures is by no means exceptional, and it would be as safe to assume that the change in the pitch of the voice of man is phylogenetically older than the sexual maturity of the ancestors of man, as it is to assume, from the same sort of evidence, that the aortic system of vertebrates is older than the mouth.

The vertebrate mouth unquestionably bears a great morphological resemblance to a pair of gill-slits. As Dohrn points out, it is bordered, like the gill-slits, by a pair of visceral arches, it lies in front of the first pair of true gill-slits, it arises at the same time with them in the embryo, and like them it opens into a section of the gut.

A ventral view of a shark shows the resemblance between the mouth and the true gill-slits in the most impressive way, and if any pair of them were to be united with each other at their ventral ends, they would become perfectly equivalent to the mouth. The armature of the mouth is repeated on the gills, and there is reason to believe that the jaw-arches have at one time carried gills like the gill-arches.

This resemblance is not imaginary. Beyond all question it is real, and it is certainly most remarkable and suggestive, but does it prove that the vertebrate mouth is phylogenetically a pair of gill-slits?

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When, in my student days, my instructor held before me the skull of a turtle and called upon me to observe the centrum, the transverse processes and the neural arch of the occipital vertebra, I was, for the time, convinced that the occipital bone had arisen by the differentiation and specialization of a bony vertebra, like those in the neck of a turtle, and that its history had been identical with that of the thoracic vertebrae, which have been differentiated and specialized in the same way into constituent parts of the bony box which covers the body of the turtle, as the skull covers the brain.

In all these cases the morphological resemblance is undeniable, but our opinion of its phylogenetic significance depends upon our view of the nature and origin of the metamerism of vertebrates, a question which will soon be discussed.

At present we must confine ourselves to a narrower point of view, and learn where we are led by Dohrn's opinion that the vertebrate mouth is actually a pair of gill-slits.

If the present mouth of the vertebrates was once a pair of gill-slits, the ancestors of the vertebrates must have had at that time another mouth, and during the long series of stages of development, while the gill-slits were gradually assuming the function of a mouth, food must have been taken in through both openings; for the new function of the gill-slits must have been acquired slowly alongside their old function, until the new mouth finally became so perfectly adapted for its new function that it supplanted and replaced the old one.

According to Dohrn, these considerations force us to believe that the primitive mouth of the ancestors of the vertebrates and of the tunicates was situated in the fossa rhomboidea, where an œsophagus pushed inwards to join the mid-gut, in the same way that it is joined in insect embryos by the fore-gut. This primitive mouth and its œsophagus were homologous with the corresponding organs of modern arthropods and annelids. The mouth of the modern vertebrates is then to be regarded as a secondary mouth, which has gradually supplanted and replaced the old one on account of its greater efficiency.

It follows from this, according to Dohrn (p. 56), that the "so-called larva" of the ascidians is a degenerated fish, and that all the features which show the derivation of the cyclostomes from

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the fishes show also that the process of degeneration has reached its extreme in the tunicates. The cyclostomes are held to owe their degeneracy to parasitism, and the most important element in the more advanced process of degeneration is that the ascidians no longer fasten themselves to fishes nor make use of their bodies as food, but that they fasten themselves to stones, to ships, or to the bodies of other animals which do not serve as food, such as the shells of crabs or the tubes of annelids.

The mouth (p. 57) which in the cyclostomes serves both as an organ for attachment to the skin of fishes, and also as a sucker for extracting their blood, has become converted in the ascidians into an organ for attachment; and these animals have thus lost their old mouth, which was homologous with that of the true vertebrates, and have acquired a new one which is homologous with the vertebrate nasal chamber.

The process, Dohrn says, must be represented as follows: The fishes take in the water for respiration through the mouth, but as this is used by the parasitic cyclostomes as a sucker, they have acquired another arrangement, and the water is not only discharged through the gill-slits, but is also inhaled through them, and, in the myxenoids, through the nasal passage also, which has in the tunicates become the functional mouth. The vertebrate mouth has lost its old function in the cyclostome-like ancestors of the tunicates, as these have gradually lost their parasitic habit, and have established themselves on lifeless bodies; but the original lips have remained, and they are to be recognized in the so-called sucking knobs of the ascidian larva, while the teeth of the cyclostomes are supposed to be represented by "bristle-carrying end knobs" upon the suckers.

The "so-called larva" of the ascidians is represented in almost every feature of its organization by the adult, sexually mature, appendicularia. No better example of the correspondence between an adult animal and an ontogenetic stage in the history of another can be desired, and we may feel confident that, whatever the phylogenetic history of appendicularia has been, that of the ascidian larva has been the same.

Nearly all of the students who have devoted themselves to the study of the tunicates agree in regarding appendicularia as a per-

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sistent representative of their primitive condition; but appendicularia is an active swimming organism, and I have shown that its simple structure is so well adapted to the needs of its pelagic life, that there can be no inherent improbability in the view that it owes its origin to simple pelagic influences.

Nothing whatever in its habit of life or in its structure lends the least support to the view that it is a degenerated animal, and if we accept it as evidence, we are forced to believe that, far from being the fixed and degenerated descendants of parasitic vertebrates, the tunicates are descended from free, active, pelagic animals of very simple structure and minute size.

Even Dohrn seems to admit that the ancestors of the tunicates were swimming animals, for he tells us in support of his view of the homology of the endostyle (Studien, etc., VIII, p. 62) that the ancestors of the tunicates were "obviously" free swimming animals, and therefore in the position to seize their food by hunting. "Offenbar waren sie frei schwimmende Geschöpfe und damit in der Lage, ihre Nahrung durch Jagd selbst zu packen."

If the tunicates are, as their embryology and comparative anatomy indicate, the descendants of an ancestor which was *obviously* a free swimming animal, it is surely simpler, in view of all the facts, to regard the gill-slits as perforations which were originally retained and fixed by natural selection as channels for the exit of the water which was taken into the mouth with the food, than to refer them back to imaginary segmental organs which have left no other trace of their existence in the body of any known tunicata.

Minute pelagic animals, with soft bodies bathed on all sides by pure water, do not need special organs of excretions or respiration, and it is not at all probable that the pharyngeal clefts were originally respiratory; but it is easy to understand how the channels through which the water flowed became converted into gill-slits, in accordance with the law of change of function, as the descendants of the primitive tunicates grew larger and became sedentary, and thus came to need respiratory organs.

It may be argued that the thing to be explained is not the existence of gill-slits, but their serial reduplication or metamerism. It may be held that the metameric repetition of the gill-slits of ascidians forces us to regard the ascidian pharynx as the primitive

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form, from which that of appendicularia has been produced by "degeneration." As we are told, however, by no less an authority than Dohrn (Studien, IX, p. 417, and VIII, p. 61) that the great number of gill-slits in the ascidians is due to secondary multiplication, "nachträgliche Vermehrung," this consideration need not detain us.

If the logical conditions of sound morphological philosophy admit the possibility of "nachträgliche Vermehrung," and permit us to believe that the twenty or thirty pairs of gill-slits which are found in ascidians are to be traced back to the eight pairs which the primitive fishes are said to have possessed, the same logic will surely permit us to believe, on sufficient evidence, that they have arisen not from eight but from a single pair like those of appendicularia.

All the vertebrates have a peculiar organ known as the thyroid gland, and while it holds no prominent place in our general conception of a vertebrate, this gland is actually one of their most constant and characteristic organs.

In all the jawed vertebrates, from the sharks up to man, its typical structure is adhered to so closely as to prove that the gland as it exists in man is an organ of vast antiquity. In all these animals it is a ductless gland, situated far back in the throat, behind the hyoid skeleton; but at an early stage in its ontogeny it is a part of the endodermal epithelium of the pharynx, and it arises on the middle line just within the mouth.

Its function in the jawed vertebrates is problematical, but these two features in its ontogeny seem to show that far back in the remote past, before it had assumed its characteristic form, it had another function which stood in some direct relation to the mouth.

The tunicate endostyle is a conspicuous organ which attracts the eye of all observers, but its true structure was first demonstrated by Fol, who proved that it is a pharyngeal gland with excretory cells to produce slime, and with ciliated cells to drive the slime out through the long, narrow, slit-like duct into the pharynx. Fol also showed its true relation to the ciliated peripharyngeal bands and dorsal lamella, and proved by simple but conclusive experiments that these organs are co-ordinated parts of a single system, which has for its function the capture of the microscopic floating food which enters the mouth with the water.

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W. Müller was the first to point out the homology of the tunicate endostyle with the vertebrate thyroid gland, and this homology has been established beyond the possibility of doubt by Schneider's discovery that the thyroid body of ammocœtes is a slime gland with an opening into the pharynx near the mouth, and that on each side of this opening a ciliated furrow or peripharyngeal band runs upwards on the inner wall of the pharynx, just in front of the first gill-slit, to its dorsal middle line, where the two unite to form an epipharyngeal band or dorsal lamella which runs backwards to the œsophagus. Even more conclusive proof of this homology is afforded by Dohrn's account (Studien, VIII) of the histological structure of the pharyngeal gland of ammocœtes, for his studies show on the one hand a most complete fundamental identity with the very peculiar and characteristic histological structure of the tunicate endostyle, and they also, on the other hand, prove its identity with the vertebrate thyroid gland, by showing that, as development progresses, it is cut up by ingrowths of connective tissue into the isolated follicles which are so characteristic of the thyroid gland. Still further confirmation is furnished by Dohrn's discovery in the torpedo embryo (Studien, VIII, p. 60) of two endodermal grooves which run from the ventral margins of the spiracles to the ventral middle line of the pharynx, to end at the median unpaired thyroid invagination in such a way as to prove that they are rudimentary peripharyngeal grooves.

This most remarkable homology can no longer be questioned. The simplest explanation, and the one which first presents itself, is the one which Müller advances, that the common ancestor of the tunicates and of the other chordata, possessed this system of organs in the form in which we now find it in the tunicates, and that while all the jawed vertebrates have inherited the ventral pharyngeal gland, it has been turned in them to some new use, as yet undiscovered by the physiologists, and has lost its primitive connection with the pharynx and its functional relation to the mouth, and has become a ductless aggregation of follicles far back in the throat.

I have tried to show that the structure and anatomical relations of this system of organs in the tunicates are quite consistent with the view that it was originally acquired for the purpose which it now serves, the capture of food.

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The simplest explanation of its origin is that which attributes it to the preservation by natural selection of a long series of slight changes, each of which improved the adaptation to the simple conditions of primitive pelagic life.

Dohrn disputes this position, and says that "many persons would have great difficulty in believing that this simple mechanism is primitive" (Studien, VIII, p. 62). The future must show how many of these persons there are, but I shall now lay before them Dohrn's own explanation, that they make comparisons for themselves.

"We ask," he says (p. 62), "how the ancestors of the tunicates obtained their food before the endostyle was formed. Obviously they were free swimming animals, and therefore in the position to seize their food by hunting. It is as certain that they needed other contrivances than the ciliated furrows and the slime-gland, as it is that the ancestors of the cirripeds sought food in some other way than by the formation of little vortices to sweep into their mouths everything within their influence. The limbs of the swimming forefathers of the cirripeds were certainly different from the cirri of modern barnacles; even so were the ancestors of the tunicates differently constructed from the modern ones, and before the slime-gland and the ciliated grooves became the exclusive means of nutrition, they must have been the accessory aids to some more primitive mode of capturing food" . . .

"Ammocœtes lives in the sand, into which even the youngest larvæ bore. Although direct observations fail, it must be assumed that the excretion of slime and the ciliation have some advantage in the nutritive or respiratory functions of organisms which live in the mud. May we not believe that, in spite of all the sifting through the oral tentacles and the velum, the hard particles of sand would be injurious to the delicate epithelium of the gut, if this were not protected by a thick coating of slime; that the ciliated furrows are adapted for conveying this slime to the most exposed parts, and that, in this function, they have their starting-point? Once brought into existence, it is not remarkable to see these useful structures further evolved until the whole mass of food is invested with a slimy admixture to facilitate its passage through the gut. It is not impossible that besides acting mechanically as an invest-

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ment, the slime also acts chemically as an aid to digestion. If this is the case, it is easy to understand how a peculiarity so useful to sedentary animals like the ascidians, or to floating ones like the salpæ, gradually assumed the whole function of nutrition. Thus the problem of the change of function is solved."

Although it seems as if the delicate walls of the gut of a burrowing animal would be more effectively protected if slime were directly excreted "upon the most exposed spots," than by this highly specialized system of organs, we might yet believe that the system "has its starting-point" in the habits of ammocœtes, if we did not find in the structure and embryology of every chordata animal which is known to exist evidence of descent from an ancestor in which it had attained, not a starting-point merely, but its full development.

The ontogenetic evidence that the vertebrate thyroid body was at one time a pharyngeal gland opening just within the mouth, and the discovery by Dohrn of rudimentary peripharyngeal grooves in the torpedo embryo (Studien, etc., VIII, Plate H, Figs. 7f, 7g, 7h and 7i), seem to me to be convincing proofs that the organs did not have their starting-point in the habits of ammocœtes nor in any degenerated fish, but that they arose in a lineal ancestor of the selachians and of the higher vertebrates, which was also an ancestor of the tunicates and cyclostomes.

Passing now from the biological relations of the system of the endostyle to its homologies, we are told by Dohrn that it is equivalent to two pairs of gill-slits; that these gill-slits were present and functional in the fish-like ancestors of the cyclostomes and tunicates, and that two of them, the mandibular clefts, moved downwards and met on the ventral middle line to form the thyroid gland or endostyle, while the endodermal portions of the others, the spiracular clefts, lost their connection with the exterior and became converted into the peripharyngeal grooves (Studien, etc., VII and VIII).

Homologies are expressions of genetic relationship, and Dohrn tells us (p. 79) that they are valuable in phylogeny only as they furnish us with the opportunity to pass from the consideration of the structure of organs as they now exist, and of the functions of these organs at the present time, to the consideration of conditions

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which have passed away; to the study of the history of the modifications which have come between these structures and functions, and those which we must attribute to the same organs at an earlier genealogical stage.

I regard the structures which we find in the tunicates and in ammocœtes as primitive, and as homologous with those which we find in the jawed vertebrates; and I have tried to trace the history of the modifications which have come between these structures of modern vertebrates and those which we must attribute to the same organs at an earlier genealogical stage in the primitive history of the ancestral pelagic chordata. The reader must judge of my success.

Let us now see what light Dohrn's homology throws on the history of these primitive modifications. He tells us (Studien, etc., VII, p. 47) that he will point out, further on, the significance of the changes which have led to the fusion, on the middle line, of structures which were originally paired; but I have been able to find nothing more upon this point except the acknowledgment, on page 63, that "I frankly admit that I have at present no available argument to bring the peculiar organization (of the ciliated grooves) of ammocœtes from a pair of imperforated (spiracular) gill-slits, into accordance with the concept of change of function; and that the origin of the slime-gland of ammocœtes from two ventrally fused (mandibular) gill-slits must for the present remain an unsolved problem."

Whatever may be thought of my own view, it must be admitted that Dohrn's homology of the endostylic system with two pairs of gill-slits has very little phylogenetic value, even when measured by his own test: the opportunity it furnishes for passing from the structure and functions of modern organs to the history of earlier genealogical stages.

Dohrn's memoirs upon the thyroid body are full of interesting anatomical details, such as the similarity between the thyroid body of the shark embryo and the true gill-slits, in their relations to the cartilages, to the muscles and to the blood-supply (VII, p. 44); and the resemblance between the peripharyngeal grooves of ammocœtes and the spiracular gills of selachians (VIII, p. 55); but as he admits that the annelidian hypothesis leaves the origin of the endostylic structures of tunicates an unsolved problem, our subject,

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the history of the tunicates, does not require us to enter into the discussion of these complicated details of vertebrate morphology.

The considerations which I have presented will undoubtedly be met by the assertion that while the simple and direct origin of the tunicates seems plausible so long as we confine ourselves to these animals alone, such a restricted view is unscientific. I shall no doubt be told that we are forced by more fundamental evidence to believe that the body cavity of the chordata is, in ultimate analysis, a segmented enterocœl formed from a series of pairs of gut-pouches, and that the simplicity of appendicularia cannot be primitive, inasmuch as the ancestors of the tunicates once possessed these complicated structures.

The first step to take in discussing this objection is to learn whether there are any traces of gut-pouches in the tunicates.

Seeliger (p. 9) has given us a very minute and detailed account of the history of the mesoderm in the clavelina embryo, and has shown that it arises from two rows of endoderm cells which give origin, in the tail, to the caudal muscles and, in the body, to free mesoderm cells which multiply with great rapidity and wander everywhere through the body cavity, which is bounded on one side by the endodermal wall of the gut, and on the other by the ectoderm.

He says emphatically (p. 128) that the mesoderm arises as two *totally unsegmented* rows of cells, each forming a single layer; that the body cavity is not an enterocœl, but a *primary body cavity;* and that the ontogeny of the tunicate mesoderm gives no evidence of derivation from paired pouches comparable to the cœlomic pouches of amphioxus.

It is a rare thing for students of tunicate morphology to agree, but in this case the phenomena are simple, and Davidoff (p. 16) completely confirms Seeliger's observations, so far as they bear upon the question, by his own studies of clavelina and distaplia.

His account of the origin of the mesoderm differs from Seeliger's in only one minor point, which has no bearing upon the question under consideration. Like Seeliger, he derives the mesoderm from two rows of endoderm cells, but he says that these cells remain as endoderm cells after they have given rise to the mesoderm, while Seeliger states that they become converted into the mesoderm.

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In all other respects Davidoff's observations are a complete confirmation of Seeliger's, for he says (600) that in distaplia the mesoderm of the caudal region persists as a solid rudiment and becomes the muscular layer of the tail, while elsewhere it breaks up into wandering mesenchyma cells. "It is to be particularly emphasized that in no part of the mesoderm is *any trace of segmentation* to be discovered, and that there is not the least indication of any cavity comparable to a myocœl. The embryonic history of the mesoderm of distaplia cannot be referred back in any way whatever to anything comparable to Hertwig's conception of the enterocœlomata."

Of clavelina he says (607): "There is not even a transitory division of the mesoderm into a somatopleur and a splanchnopleur. Even where the mesoderm is two-layered, so that a parietal and a visceral layer may be distinguished, there is no homology between these layers and the bounding walls of the cœlom of the enterocœlomata."

After reviewing all the literature on the subject, he gives as the general result of his studies the statement (p. 622) that "the body cavity of the ascidians lies between the two primary germ layers and must be regarded as a blastocœl, which would be identical with the segmentation cavity if this were not temporarily obliterated during gastrulation by the contact of the ectoderm cells and endoderm cells."

While the salpa-embryo is very complicated and unfavorable for studying this question, my own observations, which have already been described, seem to show that the body cavity of salpa is, like that of clavelina and distaplia, a primary one, fundamentally identical with the segmentation cavity, and that the mesoderm arises as free mesenchyma cells derived from the endodermal blastomeres.

The body cavity of the salpa-embryo is identical with the space between the somatic and visceral layers of follicle cells, and while there is a stage in which these two layers are in contact, the follicular cavity is undoubtedly the same as the cavity shown at *15* in Plate XI, Fig. 3, of my memoir on salpa, and this is the same as the space which is shown in Plate X, Fig. 3, between the segmenting egg and the follicle.

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In the chapter on the significance of the salpa-embryo I have given my reasons for believing that this space is homologous with the segmentation cavity of more normal tunicate embryos, and if this view be correct the body cavity of salpa is not an enterocœl but a primary body cavity or blastocœl. The mesoderm of salpa consists of free migrating cells, and the chamber of the heart is part of the body cavity, so that these cells pass through it; and while salpa is a peculiarly unfavorable subject, my observations are in complete accord with those which Seeliger and Davidoff have made under simpler and more favorable conditions.

No student of the embryology of tunicates has ever described any trace of a *series* of body cavities, and Kowalevsky, the discoverer of the cœlomic pouches of amphioxus, failed to find anything comparable to them in the tunicates, although the existence of a single pair of enterocœls has been claimed by certain observers. Van Beneden and Julin (Zool. Anzeiger, 4, 1881; Bull. Acad. Belg. (3) 7, 1884; Arch. Biol. 6, 1884) believe that the anterior portion of the body cavity of ascidians arises as a pair of gut-pouches, and that its mesoderm consists of a somatopleur and a splanchnopleur, but Davidoff has shown by careful serial sections that this statement is probably based upon erroneous observations.

Salensky holds (17, 460) that the mesoderm of the blastoderm of pyrosoma consists of two symmetrically placed cœlomic pouches, and that pyrosoma is, therefore, to be placed among the true enterocœlomata. The space between the vertebrate blastoderm and the yolk is undoubtedly homologous with the enteron, but it is by no means certain that this is the case in pyrosoma, where the food-yolk is an independent acquisition; nor do Salensky's figures show, as clearly as we might wish, that the two cœlomic vesicles open into this space, and even if this is the case, we must remember that the pyrosoma-embryo is very aberrant, and that the structure of its body cavity may be a secondary adaptation to the presence of the yolk. Taken alone it certainly is not enough to prove, without corroboration from other sources, that the body cavity of the tunicate is an enterocœl.

The ontogeny and homology of the tunicate mesoderm have been recently discussed at very great length by Seeliger (11, pp. 85–104 and pp. 126–131), by Davidoff (16, pp. 592–628), and by Salensky

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(17, pp. 456–462 and pp. 468–470 and 36–46), and as those who wish can find in these papers an extended presentation of the complicated and perplexing theory (?) of the mesoderm, I have attempted to treat it very briefly.

The literature shows that there is no direct evidence whatever of the existence, at any time in the history of the tunicates, of a metameric series of cœlomic pouches, and the supposed necessity for believing that such a series existed in the primitive chordata is only another aspect of the dogma that the metamerism of the vertebrates must have been inherited from a primitive metameric ancestor.

If, as I believe, the metamerism of vertebrates is secondary, the metamerism of the mesoderm and body cavity may have resulted from the duplication of a single pair of cœlomic pouches similar to those of echinoderm larvæ, and it is quite conceivable that these may have been acquired by the ancestors of the vertebrates after the divergence of the tunicates.

If, however, future research should show that there is a pair of gut-pouches in the embryo of appendicularia, or should prove in some other way that the structures which Salensky describes are true enterocœls inherited from an ancestral tunicate, such a discovery, which is certainly among the possibilities, would be no evidence that the primitive tunicate was the degenerated descendant of an ancestor with metameric gut-pouches.

At present, however, the evidence all tends to show that the ancestors of the tunicates had no such structure, and that the presence of cœlomic vesicles in pyrosoma is an adaptation to its peculiar mode of development.

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As these chapters from my memoir on Salpa are passing through the press for the second time, I take this opportunity to refer briefly to a paper which has appeared in the meantime by Willey (Studies on the Protochordata, Q. J. Mic. Sc., Jan. 1893, pp. 317–360).

While the author seems to agree with me in rejecting Dohrn's view that the tunicates are degenerated fishes, he holds that the ascidians exhibit, during their development, certain features of resemblance to other primitive chordata which are not exhibited by appendicularia; and he believes that these characteristics prove that the ascidians are more closely related than appendicularia to these protochordata.

The features upon which he lays most emphasis are these: I. The endostyle is at first vertical and pre-oral; II. The organ of fixation is a pre-oral lobe, and its cavity is the pre-oral or anterior body cavity; and III. The first four primary stigmata of Ciona intestinalis are developed from one primitive gill-slit; the first and fourth representing the two halves of one slit separated by the precocious development of a tongue-bar, while the third and fourth are formed by constriction from the first and fourth. The six primary stigmata are actually equivalent to three gill-slits, and the innumerable branchial stigmata of the adult are formed by subdivision of the primary stigmata and not by new perforations.

I cannot believe that students of the tunicata will regard the first and second of these arguments as entitled to the least consideration. It has long been known that the endostyle of ascidian larvae is at first vertical or at right angles to the long axis and it is so figured and described by Seeliger, but the relative position of organs is so much influenced by changes in other organs that we cannot attribute a phylogenetic significance to the position of the endostyle. The mouth is formed very late in ascidian larvæ, and as the oral region of the pharynx is rudimentary when the endostyle is formed, this latter organ does not occupy its true position until the floor of the pharynx is pushed downwards by the development of the oral region. That this is the true explanation of its change of position is shown by the fact that when the mouth continues to enlarge, as it does in the nutritive zooids of doliolum, the endostyle is pushed past its horizontal position until it finally becomes turned upside down with its long axis again at right angles to the long axis of the

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body, but with its oral end below, instead of above as it is in ascidian larvæ. See, for example, Doliolum und sein Generationswechsel, by Grobbin. Plate IV., Fig. 19.

Seeliger's carefully drawn figures of the organ of attachment of the young clavelina (Jenaische Zeitschr., XVIII, 1885, Plate 4, Figs. 40, 41, 42 and 44) show that it is at first represented by three distinct and separate tracts of cylindrical ectodermal gland cells, p. 36, ventral to the place of the future mouth, and it is only necessary to read his minute account of its ontogeny, p. 36 and p. 52, to see that its embryonic history is exactly what we should expect it to be if it has been directly acquired as an organ of fixation. If the phylogenetic history of the fixed ascidians has been as I have pictured it, each successive stage in Seeliger's account of the ontogeny of the organ of fixation is a recapitulation of a useful stage in its ancestral history, and to my mind, furnishes conclusive evidence that the ascidians are the descendants of swimming tunicates.

Willey's observations add nothing to Seeliger's excellent account of the organ of fixation, and he gives no reason for holding that it is a pre-oral lobe, except that "it contains loose mesenchyma cells derived from the two lateral mesodermic bands." This is equally true of other parts of the body cavity, and there is no more evidence that the organ of fixation is a pre-oral lobe, than there is that it is homologous with the jaws and teeth of sharks.

If it is a pre-oral lobe it is a ventral one, and it cannot be compared with the dorsal one of such protochordata as balanoglossus and amphioxus.

Willey's observation on the origin of the gill-slits of ciona are most novel and interesting and they show that we may look for most valuable results from the study of the subject in other tunicates, but they are not sufficient in themselves to prove that what he has found in one species is typical for all.

It seems to me that the author exaggerates our ignorance of the subject, for while Seeliger's descriptive account of their origin in pyrosoma is very short (Jenaische Zeitschr., XXII, 1888, p. 623), his figures show that he has traced their history most minutely, step by step, both in surface views and by sections, and that, in the buds of pyrosoma, each gill-slit is an independent perforation, and

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that they are formed in succession from in front backwards, as transverse slits without any trace of a U-shaped fold. Salensky has more recently (Zool. Jahrbücher, IV, 1891) studied their origin in the primary assidiozooids of pyrosoma, by sections, and has confirmed Seeliger's statement that each slit is an independent perforation. We have pretty satisfactory evidence that this is true of doliolum also, and Barrois' observations indicate that it is true of anchinia.

The supposed resemblance between the first pair of gill-slits of ciona and a single horseshoe-shaped slit with a tongue bar, comparable to the gill-slits of amphioxus is not based upon direct observation, however, for while Willey tells us in his summary, p. 353, that "The four primary stigmata of Ciona intestinalis *are developed* from one primitive gill-slit," we find, on page 322, that "in the actual ontogeny" the two primary gill-slits "arise by independent perforations."

The Origin of the Craspedota.

This section was intended, when written, to be part of my memoir on the genus Salpa, but lack of room prevented its publication.

Another group of animals, which, notwithstanding the text-books, I regard as a product of pelagic influences, is the craspedota, or so called hydro-medusæ.

The opinion that the hydroid jelly-fish is one of the polymorphic members of a hydroid-cormus, is, like the prevailing views on the origin of the chordata and arthropods, a result from the dogma that the aggregation of units into a compound whole must necessarily be earlier in time than the high evolution of the units.

The blastostyles and machopolyps of hydroids are, unquestionably, modified hydranths, which have arisen, in a cormus, by division of labor, and our first impulse is to believe that the origin of the sexual medusæ must have been the same.

The persistency of this opinion is natural. There seem to be many proofs that the remote ancestors of the hydro-medusæ were sessile, but I shall try to show that none of them are valid.

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It may be that the pelagic habit of the acraspeda is secondary, and while the polyps, the craspedota and the acraspeda have unquestionably had a common starting point in a free pelagic ancestor, it is quite possible that the more immediate ancestors of the acraspeda were fixed polyp-like inhabitants of the bottom.

As our opinion of the origin of the medusæ has been evolved under the erroneous notion that the craspedota and acraspeda are very closely related and have travelled the same path in company, it has been assumed that, so far as remote ancestry is concerned, what is true of some medusæ must be true of all.

If the remote ancestors of the acraspeda were inhabitants of the bottom, and if their pelagic habit is a secondary acquisition, it is natural to assume that this must be true of the craspedota as well, although all authorities now agree that the swimming habit has been independently acquired in the two groups, and that the acraspeda teach us nothing of the phylogeny of the craspedota.

Of the four grand divisions of the craspedota, two, the narcomedusæ and trachomedusæ are inhabitants of the open sea, seldom found by the shore collector, and they are so active and irritable and so easily destroyed that general collections of pelagic animals contain few traces of their existence.

While the geryonids are not uncommon near the shore, it is only by oceanic collecting and by research at sea that we can form a just conception of the abundance and diversity and scientific importance of the trachomedusæ and narcomedusæ; and thus the impression has arisen that they are aberrant and exceptional, and that the more familiar leptomedusæ and anthomedusæ and hydroids are the characteristic and typical members of the group.

The hydroid-cormus is a most conspicuous and impressive feature in the life of all our common hydro-medusæ, and in many of them, as in eudendrium, it is everything, and the medusoid structure is so degenerated that its existence is a matter of philosophy, rather than an observed fact.

All these factors have combined, during the historical growth of zoölogy, to give to the hydroid cormus a fictitious value, and this result has been promoted by the fact that many specialists have devoted themselves to the systematic study of the hydra-stage alone, to the neglect of the medusa-stage.

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Still more impressive and significant is the fact that all craspedota have a hydra-stage in their life history. Conclusive evidence shows that they are all descended from a hydra-like ancestor.

This undoubted truth has been assumed to involve the belief that this common ancestor was a fixed hydroid-cormus, although this implication is by no means inevitable.

Every one now believes that the hydro-medusa is an expanded and perfected expression of the hydroid type, and that it has been evolved from a simple, hydra-like, starting point, but the writers who have most clearly seen this homology, and who have demonstrated it most conclusively have gone a step further, and have assumed that the locomotor medusa-stage has been added on to the life of hydroids *for the purpose of distributing the species*, and that it has been evolved, according to the law of the division of labor, by the gradual specialization of certain ones among the members of a polymorphic hydroid community.

This view, which seems to have commended itself to all students, and which seems to derive support from the well-known fact that the blastostyles are, actually, nutritive hydranths which have been secondarily differentiated and specialized, by division of labor, in adaptation to the physiological needs of the cormus as a whole, this view has become so firmly established that it is now regarded as a settled and closed question.

I have no hope of effecting any change in views which are so firmly rooted, but I shall now try to show that this established opinion will not stand the test of searching examination.

From the earliest times the very ancient ciliated planula stage has provided for the distribution of coelenterates, and there is no need of other means of dispersal, nor is there any reason to think that the distribution of the species is any less important now than it has been in the past; yet one of the most remarkable and noteworthy peculiarities of hydroids, is the pronounced tendency of the medusa to degenerate and to lose its locomotor habit, and to become a sessil and degraded gonophore. The view that the sessil medusa-buds of hydroids, like hydractinia, are nascent medusæ has, very properly been abandoned, and all morphologists admit that they are degenerated and that their sessil condition is secondary.

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I for one cannot believe that hydroid cormi acquired locomotor medusæ *to distribute the species*, and that then these medusæ lost their locomotor habit, and the medusoid structure which they had slowly acquired, and that they sank into sessil, degenerated, bud-like gonophores.

If this degeneration were rare and exceptional, or if it had taken place only once or twice, or if it were an adaptation to any modern change in the ocean it would not be so remarkable, but in reality it it is one of the most noteworthy peculiarities of hydroids, and it has taken place over and over again. In every group of hydroids, in the tubularians, in the campanularians, in the hydrocorals and in the siphonophores, there are species or genera or larger groups with degenerated gonophores, and there is ample evidence that this has not been inherited from a common source, but that it has been acquired again and again. Hydractinia, with its sessil degenerated gonophores is clearly related to podocoryne with its free medusa (dysmorphosa). Tubularia with medusa buds is closely related to corymorpha with its free medusa (steenstrupia). Laomedæa stands in the same relation to obelia; bougainvillia to heterocordyla and so on, and by far the best example in the whole animal kingdom of independent modification along parallel lines, is to be found in the degeneration of the sexual locomotor medusæ of compound hydroids. Among the most widely distributed genera and species are some of those in which this degeneracy is most complete, such as the species of eudendrium, hydractinia, laomedæa, cordylophora, and hydra, and I think we may dismiss the idea that the medusa was originally acquired for the purpose of distributing the species, and with it the idea that it has been produced by division of labor, which is disproved by the following facts.

The four great groups of craspedota, the narcomedusæ, the trachomedusæ, the anthomedusæ and the leptomedusæ agree with each other in the possession of a gelatinous bell, a muscular sub-umbrella, and velum, and in the presence of tentacles and sense-organs on the bell margin, and we are therefore justified in assuming that these common structural characteristics were acquired before these groups diverged from each other; that they are older than the modern genera and families, and older than the differentiation of the hydroids into tubularians and campanularians.

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Among these are some, the narcomedusæ and trachomedusæ, in which the hydra larva completes its individual development and grows up into a sexual medusa, and others, the anthomedusæ and leptomedusæ, in which there is polymorphic differentiation into hydranths, and sexual medusæ or gonophores. The presumption in morphology, whenever there is nothing which demands the contrary, is that the simple is older than the complex, and we should therefore be justified in holding that the craspedota without polymorphism, where each hydra grows up into a medusa, are more primitive than those with polymorphism, even if there were no positive evidence, and I shall now show that this view is supported by positive proof. Omitting hydra, which is too aberrant and too much isolated to be available for comparison, the hydroids are, almost without exception, larval or sexually immature, and the reproductive persons are all medusæ, or else gonophores which show by their structure that they are degenerated medusæ; even hydra itself is probably a hydroid with gonophores in an extreme stage of degradation. The affinities of hydra are doubtful, however, but in most cases we may state definitely that the hydroid is larval in its sexual nature; and all the hydroids which we know must be traced back to an ancestor with a larval hydra stage and an adult sexual medusa stage; for we must attribute to inheritance from a common ancestor all they have in common except what can be shown to be due to secondary modification.

The question of the origin of the craspedota then narrows itself down to this; was the sessil mode of life, and the habit of forming cormi which has resulted from it, acquired before or after the evolution of the medusa; was the hydra-like ancestor of the hydromedusæ, a solitary pelagic animal as a sessil cormus.

In discussing this question we must keep in mind the following considerations; first, that the history of the polyps and acraspeda has no bearing on the subject, since these animals belong to another line of descent; secondly, we have the very remarkable fact that the sex of all the progeny of a hydroid egg is usually fixed from the start, and is either exclusively male or exclusively female. The egg of a hydractinia may give rise to hundreds of hydranths and machopolyps before it produces blastostyles, and these grow up before they produce medusa buds; the number of medusa buds

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which ultimately spring from the progeny of a single egg is very great indeed and practically unlimited, and yet when these buds produce reproductive elements they are of the same sex in all the members of the colony.

This fact is very remarkable when we bear in mind the length of the path from the egg to the sexual bud, and the complicated character of the alternation of generations in hydroids.

One of the most constant results of a sedentary or fixed habit of life is hermaphroditism; and its influence in this direction is so potent that it has, in the barnacles, broken down one of the most ancient and persistent of all the characteristics of animals, the separation of the sexes of arthropods.

If the sessil habit of hydroids were primitive we should certainly expect them to be hermaphrodites and the potential unisexuality of each hydroid cormus would be unintelligible; although it is easily understood if the cormi have arisen by the asexual multiplication of the larvæ of unisexual locomotor adults, for in this case each larva, before it began to form cormi, must have been potentially either a male or a female, and all its progeny, produced by budding, would naturally inherit the same sex, so long as sex remained potential and was not called into activity, for there is no reason why the sedentary habit of larvæ should be followed by hermaphroditism in locomotor adults, nor is there any reason why the sex of larvæ should be modified by a sessil life so long as sex remains latent or undeveloped in the bodies of these larvæ during the sessil stage. It is remarkable that those species in which the gonophores are most degenerated should adhere to the same law, but this fact shows the firm hold which the separation of the sexes has taken upon the organization of hydroids, and is to my mind one of the most conclusive proofs that the sessil cormus is secondary.

In the third place we must remember that the craspedota with a sessil hydroid cormus form only a part of the group; that the trachomedusæ in which, so far as we know, the hydra larva is always a free, floating solitary animal, are numerous and diversified, and that, while the life history of the various narcomedusæ has been greatly modified by parasitism, they may all be reduced to a type in which each egg gives rise to a simple floating hydra-like larva which grows up into an adult like the larva of a trachomedusa.

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The structure of an ordinary nutritive hydranth from a cormus, with its funnel-shaped crown of numerous tentacles placed in a circle around the mouth at the oral end of an elongated body, is undoubtedly an adaptation to a life on the bottom, but there is ample evidence that this hydroid type, while the most familiar one, is not the most primitive, nor the one from which the veiled medusæ have been evolved.

Every one who has reared from the egg many species and individuals knows that the hydra which is formed from the planula, shows a marked tendency to pass through a stage with first four and then eight symmetrical, stiff tentacles, so placed that they do not form a crown, since four point forwards and the other four backwards in such a way that they radiate from the body like the pseudopodia of a pelagic rhizopod.

In the simplest and most primitive of the hydroids, the Codonidæ or tubularias, this larva often has at first, a floating habit, and a distinctive name, actinula, has been given to it on account of its star-like shape.

The number of tentacles, like the number of arms in a star-fish, varies somewhat, but they are radially arranged in two alternating sets, and in the majority of the individuals there are four primary radii.

The quadrate structure of young hydroids has been noted by Haeckel, and he points out (Ueber die Individualität des Thierkörpers, Jena. Zeitschr., XII, 1878, p. 16) that while the number of tentacles may, at first, have been indeterminate and variable, the early establishment of the number four in the organization of the ancestors of the acalephs is shown by the fact that in the young hydra, as well as in many other hydro-polyps, and in the young actinia and in many other acalephs there are only four primary tentacles.

Haeckel also says that he regards the four-fold condition as primitive for the medusæ, and that all the six rayed and eight rayed medusæ are to be derived from a form with four rays, and that the oral lobes, the primary radial canals and the primary marginal tentacles all lie in the four primary radia. He also gives his reasons for believing that the corals and ctenophores retain traces of this primitive quadrate organization.

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Now what does this quadrate structure mean? No one who has watched a pelagic hydro-medusa, as it first swims to the surface, with contracted tentacles, by rapid vigorous pulsations of its bell, and then sinks slowly down, permitting its tentacles to be drawn out by the friction of the water, into long slender threads until they form a living poisoned net, stretched like a spider's web, to entangle the floating organisms of the ocean; no one who has witnessed this can doubt the perfect adaptation of its structure to the conditions of its pelagic life. It does not, like a bilateral organism, pursue its prey in horizontal lines, but it captures it while sinking, and then rises to the surface to repeat the process, and its most important relations to space, are radial to the earth and to gravity while those of bilateral animals are concentric with the earth's surface.

We do not know enough about the biological relations of the medusæ to say what physiological superiority definite radiation has over indefinite radiation, but I think we may feel confident that the quadrate radiation of the hydro-medusæ is an adaptation to a more varied environment than that of a sessil hydra, and that it has been acquired by free animals. In fact the quadrate structure of sessil gonophores is generally accepted as evidence of their descent from free medusæ, and the advocates of the hypothesis of polymorphism attribute the acquisition of the quadrate structure to the free locomotor life of the reproductive persons of the cormus.

If we admit this what shall we say of the quadrate structure of young hydroids? We might see in the four primary tentacles the accelerated acquisition of medusoid characters if the larval hydroid were not in most cases separated from the medusa by the intervention of numerous generations of hydranths with a great and variable number of tentacles.

In the actinula of tubularia and in the cunina larva the proboscis or manubrium is long and the body is short, so that the zone where the tentacles are inserted is more nearly equatorial than it is in ordinary hydranths; and the larva, which is ciliated, is thus more perfectly adapted for a pelagic life.

I shall now show that the quadrate floating hydra and not an ordinary hydranth with an oral crown, is the ancestral type from which hydro-medusæ have arisen.

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The craspedota of modern times fall into two divergent groups, which must have separated from their common stem very early. In the one group are the trachomedusæ, the anthomedusæ and the leptomedusæ; agreeing in the possession of circular and radial chymiferous tubes, differentiated out of the simple larval digestive cavity by the formation of areas of adhesion between its oral and aboral walls; and in the other we have the narcomedusæ, in which chymiferous tubes are absent and the digestive cavity is formed out of that of the hydra-like larva in a much more simple and direct way, as Wilson's account (The structure of Cunocantha in the adult and larval stages, by H. V. Wilson, Studies from the Biol. Lab., Johns Hopkins Univ.) which has recently been corroborated by the researches of Maas (Ueber Bau und Entwicklung der Cuninen-knospen. Zoologischen Jahrbüchern, V, 272) shows.

While the lines of descent represented by these two types are quite distinct we cannot doubt their origin in a common ancestor from which both the hydra-like larva, and the bell, sub-umbrella velum, and marginal tentacles, sense organs and nervous system of all the craspedota have been inherited, neither can we doubt that this primitive veiled medusa was still more primitively derived from a hydra-like form.

Now the fact that the sessil funnel-shaped hydra is restricted to a part of the members of one of these primary subdivisions of the craspedota, while the solitary star-shaped floating hydra-like larva is found in both of them; in the larva of cunina, as well as in that of the geryonids, and in the actinula of tubularia, shows that the ancestral form which the hydra-larva represents was of the latter type, and that the more familiar hydranth is a secondary modification of the more simple and ancient form.

In a more extensive discussion of this subject (The Life History of the Hydromedusæ, Mem. Boston Soc. Nat. Hist., 1886) I have referred to the history of parasitism and its effects, in the narcomedusæ, in order to show how easily this primitive larval type may become converted into a polymorphic cormus with alternation of generations. Nothing could have been further from my mind than a belief that the cormi of ordinary hydroids have been phylogenetically derived from the parasitic cormus of cunina larvæ.

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The knowledge of the true structure and mode of origin of the adult cunina which we owe to Wilson's researches, already referred to, proves that the narcomedusæ represent an independent and very primitive branch of the craspedota and while I was ignorant of this at the time my memoir was written, and while I then, with all other naturalists, believed the narcomedusae to be much nearer to the hydroids than they really are, I recognized, and thought that I had clearly stated my opinion, that the cormus and the alternation of generations in parasitic cuninas is a secondary and independent acquisition, although I still think, as I did then, that the analogy is most suggestive and instructive, inasmuch as it shows how easily secondary complications, essentially similar to those of hydroid cormi, may be grafted on to the structure of a simple hydra.

I regret that I am not able to refer to my memoir as I write, for critics of my views have spoken as if I regarded the parasitic cunina-cormus, as the ancestor of modern hydroids. Thus, for example, Maas says, p. 295, that Metschnichoff and Brooks hold that the narcomedusæ are primitive forms, *and that the alternation of generations of the hydroid polyps is derived from that of cunina.*

As far as I myself am concerned, I certainly regard the unmodified cunina metamorphosis as primitive, but I regard the complications which have been introduced by parasitism, as analogous to, but quite independent of, the peculiarities of hydroids.

A general view of a subject so full of complicated details as the life history of the craspedota is difficult, as illustrations and minute accounts of specific instances are so necessary. I hope, however, that, notwithstanding Lang's verdict (Ueber die Einfluss der festsitzenden Lebensweise, p. 159) that it is "the most improbable of improbabilities" the view that the craspedota owe their origin to pelagic influences will commend itself to those who are most familiar with these animals.

It is hardly probable, however, that our modern craspedota are primitively pelagic, for their size, the complication of their organization, and especially their great diversity of structure and habits, would seem to show that while they have been evolved in the open ocean, they are not the product of primitive and simple conditions, but that they show the influence of the more intense and complicated struggle for existence which has come from competition with

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animals which, having been evolved at the bottom, have then become secondarily pelagic and have taken their improved structure back into the open ocean.

Haeckel has shown that the highest representatives of the craspedote stem, the Disconectæ (velella, porpita, etc.) and the siphonophores, are pelagic productions, and that the Disconectæ can be traced back to an ancestor similar to the Trachomedusæ, while the Siphonanthæ or true siphonophores have arisen from simple Anthomedusæ. (Report on the Siphonophora collected by H. M. S. Challenger; and System der Siphonophoren, Jenaische Zeitschrift, Vol. XXII, p. l. 1888).

Both these groups are therefore pelagic in their history, and they go back, not to ancestral hydroid cormi, but to ancestral medusæ, but they can hardly be primitively pelagic, and we must regard them as the product of the more modern conditions of pelagic life.

The craspedota were undoubtedly represented in the primitive pelagic fauna, by floating hydras with stiff radiating poisoned pseudopodia-like tentacles, and also by small and simple veiled medusæ, but the higher forms of the group are probably more modern, although there is palæontological evidence that they are as old as the lower cambrian.

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AN UNDESCRIBED ACRANIATE: *ASYMMETRON LUCAYANUM.*

By E. A. ANDREWS. With Plates XIII and XIV.

While the Johns Hopkins Marine Station was located at Alice Town, North Bemini, Bahamas, in June and July, 1892, a small amphioxus or lancelet came under observation and was at once recognized as quite different from those commonly known.

Owing to the very great morphological interest attached to our knowledge of the acraniate vertebrates, this new member of the group has been studied and compared with the known lancelets to determine to what extent it adds to the present conception of acraniate anatomy. The result, as presented in the present article, is that this Bahama lancelet is generically distinct, though morphologically but little removed from the others in the main features that make the acraniates isolated from all higher forms.

Before stating what are these generic characters the habits and habitat of the living animal, as far as known, may serve as a hint of the possible explanation of some of those anatomical peculiarities.

Examination of the very strong current that passes out from the lagoon to the Gulf-stream between North and South Bemini frequently resulted in the capture of large numbers of these small lancelets. They were taken in the tow-net while swimming at or near the surface; most abundantly at the early part of the ebb-tide when it had been high tide about nine o'clock in the evening; rarely in the daytime or late at night or on the rising tide. They were also obtained buried in the sand-flats that furnish the characteristic fauna of this ebb-tide current, but only a few could be found here and there at the western end of Stokes Cay and also half a mile to the east of East Point, East Wells. They may, however, be much more abundant in these flats than would appear from the above statement, since their small size and the soft permeable nature of the purely calcareous sand would make their detection much more difficult than it would be for such large lancelets as *Branchiostoma caribæum* which we had taken in great numbers in the firm continental sands of Tampa Bay, Florida.

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The specimens taken in June were larger, often sexually mature, while those taken later were generally immature or larval forms.

In captivity they live like the European lancelet; occasionally leaving the sand and swimming about with considerable velocity, but soon falling to the bottom and resting upon one side as if exhausted or else burrowing instantly into the sand within which they move about with great celerity. As long as the water be pure they rarely leave the sand in the daytime and then quickly return when stimulated to motion by a slight touch or jar.

In flat glass dishes without sand they arrange themselves with reference to light in a manner suggesting that we have here amongst these simple, eyeless vertebrates an example of heliotropic movement such as plants and many non-vertebrates exhibit. At the end of some hours of alternating periods of active locomotion and lethargic rest most of the individuals are found collected upon one side of the dish, that furthest from the light.

The same distribution ultimately results when the dish is turned through 180°. When carried to the door of the laboratory and exposed to the direct rays of the sun a most violent commotion immediately followed and soon resulted in the collection of all the passive individuals upon the side of the dish most remote from the source of light.

Finally when the dish was placed in the centre of a small square room lighted by a window on the north and one on the east and either window opened, the animals collected in the part of the dish most remote from that window. When both windows remained open the position of the lancelets was vaguely that part of the dish most remote from both windows.

As far as these experiments go they may be taken to indicate that we have a case of negative heliotropism in these simple vertebrates: that the lancelets move away from the source of light along the direction of its rays: that the directive action of the sun's rays is as in the numerous non-vertebrates studied by Dr. J. Loeb.

Though small, the largest not exceeding a length of 16 mm., many were sexually mature, yet individuals kept for many weeks did not discharge ova, except in one collection taken early in June; in this case some of the ova were naturally fertilized and a few developed as far as the gastrula stage, when they were destroyed by an accident.

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As the animal is small and translucent the course of food and carmine granules may be traced through most of the digestive tract. The strong current of water setting into the pre-oral chamber seems to be controlled by the longitudinal, ciliated ridges of Müller's "Räderorgan" which pass forward from the velum on the inside of each lateral wall of the pre-oral chamber. Thus the carmine granules pass rapidly along these ridges towards the velum where they are turned inward towards the aperture, mouth, at the centre of the velum. The granules pass along in strings as if held together by a thin mucous; once through the mouth they do not pass out through the pharyngeal slits but continue along the median dorsal pharyngeal groove, the hyper-pharyngeal groove, still adhering to one another in strings. In actual longitudinal and cross sections these strings of granules may still be recognized in the ciliated hyper-pharyngeal groove.

Explanation of diagram. The dotted line indicates the course of food passing through the digestive tract which is divided into pre-oral chamber, pharynx, stomach with diverticulum and first and second intestinal regions.

This groove leads into what may be called the stomach within which the current of granules, or the granule containing mucous strand, turns abruptly downwards and forwards as indicated in the above diagram, yet does not enter the diverticulum but, still under the control of the active cilia lining the digestive tract, passes back again through the centre of the stomach. It is now revolving rapidly in a constant direction from right to left and continues to do so throughout the next division of the digestive tract, the first or larger section of the intestine. In this part of the intestine the granules, diatomes, etc., collect into clumps separated by the clear part of the continuous mucous-like strand. In this region the intestinal epithelium is especially modified in a zone that became very prominent when staining reagents are used.

The second, final and smaller part of the intestine, contains pellets of detritus that become successively larger towards the anus where

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they have the form of elongated, not spherical masses that are discharged from the anus as the anal sphincter relaxes from time to time. The mucous strand is here broken, the granular aggregates becoming isolated from one another. These balls or pellets move along but slowly and give up the revolving motion seen in the first part of the intestine and in the stomach.

The time elapsing from the addition of carmine to the water to the discharge of carmine containing pellets may be much less than one hour.

Though no carmine was seen to enter the diverticulum, yet after twenty-four hours its walls became pink from the accumulation of fine granules that, in sections, are seen to lie within the epithelial cells of this part of the digestive tract. When the animal is kept long in sand and water to which carmine is added from time to time, this is taken up by many cells on the branchial arches as well as by the cells of the stomach and intestine. Moreover, as demonstrated by Weiss (**24**) for the European Amphioxus, many cells of the atrium take up the carmine, notably those forming the so-called nephridial ridges and those in the pigmented, dorsal atrial pouches. The nephridia, however, could not be recognized in sections of such individuals.

After some weeks the pre-oral organ was found darkly stained by the carmine, but not uniformly so: the closed deep lying part contained most of the red in the form of radiating spherules as if in gland cells while the tubular part of the organ opening into the pre-oral chamber had a decidedly yellow color with only diffused red in the free ends of the cells and very few red granules in their basal parts. This appearance of the pre-oral organ suggested at the time that it might have a nephridial function.

When Bismarck brown is added to the water in which the live animals are kept, they soon became darkly stained. On sectioning, this stain is found to be due to fine spherules in the cells of the epidermis, digestive tract, atrium and testis and also in the cells lining lymph spaces. Here again the method failed in revealing nephridia.

The vitality of injured specimens is very great; even those cut into two live for some days. In such cases some regeneration of lost parts may be initiated. Thus when the tail was cut off just

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posterior to or at the anus, a blunt process grew out from the healed-over wound. On examination by sectioning, this process was found to contain notochord, anus and nerve tube and to be covered by a complete new epidermis. Here, as in the tadpole or salamander, there seems to be a regeneration of each tissue to form new tissue of its own kind. The epidermis was continuous with the old one and underlaid by a delicate connective tissue lamella continuous with the much thicker one under the old epidermis. The nerve tube was a collection of a few large cells continuous with the walls of the old nerve cord and surrounding a relatively large central lumen. The notochord had grown out very much as in the figures of Siredon given by Barfurth and was enclosed in a delicate sheath continuous with the stout sheath of the old cord. The digestive tract was continued to the surface of the body where its lining cells became continuous with the outer epidermis. The muscles of the last pair of myotomes appeared to be broken down into an amorphous mass that was continuous with a new mesodermal formation in the new tail. This mesoderm mass consisted of a loose sponge-work of cells arranged in the proximal part of the new growth so as to surround segmentally-placed cavities. Large blood, or cœlomic, spaces full of coagulum or lymph also occurred in this part of the new outgrowth.

With sufficient care the specimens might well have regenerated complete post anal regions—as far as could be surmised from these few observations.

Coming now to the chief anatomical peculiarities of this new Acraniate we find them expressed in the asymmetry of the reproductive organs and in the character of the fins.

In the living animal the reproductive organs, ovaries and testes, may be easily seen through the body-wall, but much more readily from the right side (Fig. 1) than from the left (Fig. 2). This is due to the fact that the gonads form a single series present only upon the right side of the animal and are not paired as in all other Acraniata and in most all Craniata. From the right this single series of gonads shows plainly through the wall of the atrium while from the left it is more or less concealed by both sides of the pharynx, the left wall of the atrium and, in part, by the caecal pouch that lies on the left of the animal.

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This is obvious in transverse sections (Fig. 14) where the gonad, in this case an ovary, though having the normal relation and position of those found in a common Amphioxus and so developed as to crowd the digestive organs out of place, is yet found only upon the right side. Longitudinal sections show the entire series of gonads all upon the right side so that there is no question of alternate crowding of left and right gonads into a single series. Both testes and ovaries alike exhibit this asymmetrical development, though otherwise like those of other lancelets.

That this single right series corresponds to the right series of Amphioxus without admixture of the left is indicated by its presence as a single right series in the youngest individuals observed. Thus in a specimen 6 mm. long (Fig. 5) the gonads are a single, right series of minute collections of few large cells, the sex as yet unrecognizable, having the same position and character as the early stages of the gonads on right or left of the common Amphioxus as figured by Boveri (**25** Fig. 8).

It is thus evident that the asymmetry of the reproductive organs goes back to an early stage and is probably due to the lack of development of the left series found in other Acraniata. Whether there is at the first any start of this missing left series cannot be determined at present.

The gonads differ from those of the known lancelets in being somewhat more numerous on the right side. Thus while the European form has according to Lankester (**23**) but 26 gonads on the right (and as many on the left) this Bahama form has 29 as seen in Fig. 1. They begin at the fifteenth myotome, gradually increase greatly in size, become consequently crowded together (since each corresponds to a myotome), rather suddenly decrease in size posterior to the branchial region and end anterior to the atriopore by a very small gonad like the first of the series, lying in the forty-third metamere.[1]

No observations were made upon the method of discharge of sexual products, but in sections ova and sperm are found in all parts of the digestive tract, in the pre-oral chamber, stomach, intestine and extreme end of the rectum. In some cases the ova in the

[1] In Fig. 1 the correspondence between myotomes and ovaries has not been correctly represented.

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intestine show the first cleaveage spindles, having been fertilized. As all these cases were specimens kept in confinement we infer that these sexual products were probably swallowed and give no evidence as to the method of discharge from the gonads. None were found free in the atrium; and in some cases the individual with ova in its digestive tract was sexually immature.

Even more conspicuous than this asymmetry of the reproductive organs is the character of the fins at the posterior end of the animal.

As seen in Figs. 1, 2, 4, there is a long slender caudal process extending posterior to the last myotome. This process is composed of the rather low dorsal and ventral median fins which become continuous at its tip but are elsewhere separated by the supporting notochord that extends nearly to the very tip of the caudal process. Moreover the nerve cord runs back in this caudal process as a slender tube just dorsal to the notochord and finally diminishing to a few cells surrounding a lumen at the extreme tip of the process. In a transverse section (Fig. 23) taken about the region *h* of Fig. 4 the dorsal and ventral fins are almost identical in size and structure. Each is a clear matrix of connective tissue permeated by irregularly radiating, anastomosing canals lined by cells that may nearly fill them, as represented to the extreme right in Fig. 25. This clear substance is concentrated as a sheath about the notochord and serves peripherally as a support for the single layer of epidermal cells covering the caudal process as all other parts of the body. The minute nerve cord has ganglion cells but no pigment. The numerous nerves branching through the caudal process come for the most part, if not altogether, from the neural tube anterior to the caudal process.

In the youngest forms found, having a length of 6 mm. and 22 branchial clefts on a side, the caudal process is very much shorter, not narrowed, but on the contrary expanded as the characteristic, rounded, larval tail-fin (Fig. 5). Soon, however, this rounded tail-fin becomes pointed, as seen in a specimen with twenty-seven branchial slits (Fig. 3), and subsequently elongated as in the sexually mature individuals.

Where the caudal process springs from the myotome region the ventral and the dorsal median fins both suddenly increase in height, the ventral much more than the dorsal.

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If we now trace the dorsal fin anteriorly in the region of the posterior myotomes we find that the lymph canals that penetrate its substance become arranged in definite, parallel lines radiating upward from near the neural cord. Several of these canals occur opposite each myotome and some connect with large lymph spaces now appearing just above the neural cord. As seen in Fig. 25, a surface view from an animal living in sand with Bismarck brown, granules of brown substance occur in large cells lining the canals and the lymph cavities. These cavities become larger and more regular anteriorly and form the fin-ray spaces of the dorsal fin, three or four for each myotome. Henceforth the dorsal fin becomes more and more predominantly made up of these lymph spaces which succeed one another in a constant crowded series as seen in Figs. 1, 2, 3, 4 and in Fig. 17.

The lymph canals still remain and at places open into the fin-ray spaces, from the summits of which they extend out into the fin. Though at first nearly vertical (Fig. 25) these canals bend backwards more and more until, anteriorly, they come to run nearly parallel to the dorsal edge of the fin and will appear in transverse sections as in Fig. 22.

The fin-ray spaces are lined by a membrane with distinct cells projecting into the lumen (Fig. 17). Though at first quite near the nerve cord the spaces gradually rise, anteriorly, as the muscles become vertically deeper, and thus come to be separated from the nerve cord by a considerable space as seen in Figs. 22, 14, 12.

Projecting into each well formed fin-ray space, but not into the imperfect posterior ones represented in Fig. 25, there is a ventral mass of solid connective tissue, the so-called fin-ray. These fin-rays (Fig. 17) vary much in size and shape in differently prepared specimens but never appear as prominent as in some species of Amphioxus. They are not paired but single, median upgrowths from the ventral wall of each fin-ray space.

About the middle of the branchial region the proportions of the fin-rays and fin-ray spaces are as in Fig. 17, but posteriorly they became more crowded and deeper vertically, most so over the region of the atriopore and just posterior to that region. Anteriorly the spaces become more shallow and longer till finally the fin-rays cease to exist over the pre-oral chamber while the fin-ray spaces

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continue as flattened cavities. Anterior to the pre-oral chamber only a few very small irregular median spaces remain as representatives of the fin-ray spaces. As seen in Fig. 10, the most anterior of these opens into a large unsegmented, median space that comes down abruptly into proximity with the brain, where it ends dorsal to the pigment spot that limits the anterior end of the brain.

As is evident from the figure this space opens into at least one of a series of long lymph canals passing back dorsal to the fin-ray spaces in the substance of the dorsal fin. Such canals are seen cut in cross section in Figs. 1, 3, 15, 21, 22, 23. Many of these canals arise still further forward from vertical, radiating canals anterior to the brain, canals that differ from those in the posterior part of the body (Fig. 25) chiefly in having no swollen base, or incipient fin-ray space. In the anterior region there is, however, what may be regarded as a representative of an undifferentiated series of fin-ray spaces developed as a single unsegmented space lying immediately above the notochord and extending forward from the brain almost to the extreme tip of the notochord. This terminal space has no connection with the one dorsal to the brain and is hence cut off from the series of fin-ray spaces by an interval above the pigment spot.

In this interval lies a minute intermediate lymph space, as shown in Fig. 10.

Anteriorly the terminal space appears constricted into partially separate, minute spaces near the tip of the notochord. A transverse section taken near the tip of the notochord (Fig. 11) shows this terminal space and a few of the lymph canals imbedded in the small amount of connective tissue that makes up the dorsal fin.

Throughout the dorsal fin the lymph canals have a nearly vertical position at the anterior and posterior ends but elsewhere become more nearly horizontal. Those from the anterior region running backward (Fig. 10) incline more and more and diminish in number (Fig. 17), while those from the posterior end turning forward supply all the posterior part of the fin.

If now the ventral median fin be traced forward from the caudal process it will be found to present the same structure as the dorsal fin with the important exception that it contains no fin-rays nor fin-ray spaces: thus it differs from the ventral fin of other lancelets.

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The radiating vertical canals are very long in the deep fin beneath the posterior myotomes and present slight proximal swellings in the region just posterior to the anus; swellings that are like those more posterior ones of the dorsal fin represented in Fig. 25.

Anterior to the anus, however, these spaces are not continued as fin-ray spaces similar to those of the dorsal fin; in fact there are no representatives of such spaces and the lymph canals cease to form parallel, vertical channels and anastomose in an irregular way.

There are thus no double fin-rays in the region between the anus and atriopore though such are believed to exist in all other Acraniata: a striking and important difference.

At the extreme anterior tip of the animal the ventral fin has the same structure as the dorsal, with which it is continuous around the anterior end of the notochord (Fig. 10).

In this region the ventral fin has also a large median lymph space that anteriorly is somewhat sub-divided near the tip of the notochord, but posteriorly expands vertically as a large median space that penetrates some distance into the ventral fin and then rises again to end abruptly anterior to the pre-oral chamber opposite the posterior end of the second myotome (Fig. 10). In transverse section, near the tip of the notochord, this ventral space (Fig. 11) is smaller than the dorsal space, but in sections posterior to the brain it is in every way larger than the dorsal spaces above the nerve cord.

Between this anterior end of the median fin and the posterior part in the caudal process important modifications of the median fin, in fact its entire disappearance as a median fin, are brought about in connection with the openings of the digestive and branchial cavities. These modifications are the departure of the median fin from a true median position, and the substitution of the paired metapleural folds in place of a median fin throughout the length of the branchial region.

The appearances seen in the youngest specimens favor that explanation of the formation of the atrium given by Willey (**26**) so that we may adopt the terms sub-atrial ridges for the horizontal outgrowths of the metapleural folds and abandon the older idea of extensive epipleural downgrowths. The complete metapleura have the same structure and relationship as in the common amphioxus,

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starting from the pre-oral hood they run back parallel with one another as far as the atriopore, thus forming the lateral limits of the floor of the atrium. They do not stop at the atriopore, but as seen in Figs. 1, 2, 3, 4, 5, continue posteriorily, the left one but a short distance, the right one, however, becoming continuous with the median ventral fin. This continuity of the median ventral fin and the right metapleuron can be seen in surface views (Figs. 1–5), and also demonstrated in serial sections.

Such sections show that the fin retains its solid structure up to point anterior to the atriopore where it, or the metapleuron as we may now call it, acquired the usual hollow condition known in other lancelets. The left metapleuron likewise is solid and identical with a fin in structure at its posterior end. In a section across the middle of the body (Fig. 14) we see two hollow metapleura, but at the level of the atriopore (Fig. 15) there are two fins, the left the smaller. Following a few sections forward from the atriopore the thick connective mass of the fins is found to continue as the thick sub-epidermal layer on the outer side of the metapleura, while a new cavity, the large lymph space of the metapleura, gradually extends downward from the basal part of each fin, or metapleuron.

This continuity of the right metapleuron with the median fin is unlike the condition found in the European Amphioxus and leads to an interesting departure of the fin from its true median position. In passing forward from the tail process the median fin departs decidedly to the right, even before the anus is reached, and becomes attached along the right edge of the ventral aspect.

Where the anus opens out on an anal papilla (Fig. 22) the fin is on the right side. We see here that though the digestive tract discharges to the left of the median fin it is not at all asymmetrical with reference to the median plane of the animal: it is the fin which is placed on the right, while the digestive tract remains a median structure. Yet the actual orifice of the rectum may, as in the figure, be turned to one side and lie to the left of the median plane; the papilla with its sphincter muscles is not accurately bisected by the median plane, but this does not justify us in regarding the anus as morphologically out of the median plane.

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Immediately anterior to the anus the rectum is median (Fig. 21) and the fin is on the right side; but before the atriopore is reached it approaches the median line and appears as a true median structure as far as its free portion is concerned. The basal part, however, even when the free edge is median, extends up to an attachment to the muscular region of the right, much as is seen in Figure 21. Even its free part is median only for a short space and soon projects again from the right side, becoming thus continuous with the right metapleuron posterior to the atriopore, as is shown in Fig. 15, where the left metapleuron is seen in a similar fin-like state and the posterior tip of the atrioporal spout is enclosed between the two.

The departure of the median fin from a true median position is correlated with the breaking through of the median rectum: anterior to the anus the fin becomes more nearly median in proportion as the rectum rises up from its superficial ventral position or is more and more enclosed by the downward extension of the lateral muscles, passing from the opening seen in Fig. 22 through a stage shown in Fig. 21 to a position shown in Fig. 15. Here, however, the atrial formations again interfere with the median position of the fin, and it becomes lost in the right metapleuron.

The continuity of right metapleuron and fin suggests that the fin may not be primarily a median structure, but one of a pair of longitudinal folds of which the left forms only the left metapleuron while the right is more extensive and comes to lie in the median plane, as a locomotor organ, whenever the atrium and digestive tract are not at the surface to render such a position difficult.

The idea that the median fin is but a form of the right metapleuron would not harmonize with the view suggested by Lankester (**23**), that the double ventral fin of amphioxus may be the fused epipleura, but that view would require re-stating if we accept the formation of the atrium as recently described (**26**).

As we have seen the median ventral fin of the caudal process continue around the end of the notochord, thence along the whole dorsal median line and finally become continuous with a median ventral fin beneath the anterior end of the notochord, nowhere showing any trace of a double or paired character, we might expect that it would become continuous with the right metapleuron

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anteriorly as well as posteriorly. According to Lankester (**23**) this is the case in amphioxus if we regard the right half of the pre-oral hood as a continuation of the right "epipleuron." But in the Bahama amphioxus both metapleura seem to be continuous with the side of the hood, these with the edges of the anterior part of the pre-oral chamber and these in turn with the single median fin (Fig. 6).

In Fig. 13, which is a section across the base of the hood, the connective tissue mass joining the shrunken-in hood membrane to the lateral muscle mass of each side is a direct continuation of and is some sort a representative of the metapleuron of each side. Again in the section Fig. 12, anterior to the free edge of the hood the same connective-tissue masses now project freely and form edges to the pre-oral chamber. They are much like the fins, or metaplura, posterior to the atriopore (Fig. 15).

Even if we may thus regard the metapleura as continuous with the sides of the pre-oral hood and the anterior edges of the pre-oral chamber, we are puzzled by the fact that they meet at the anterior end of the pre-oral chamber and became continuous with the median fin (Figs. 6, 20). At the same time the right predominates here also and it is chiefly the right side of the pre-oral chamber which is fringed by a continuation of the median fin. As the median fin is thus posteriorly continuous with a single paired structure, the right metapleuron, and anteriorly continues with what appears to be two paired structures, both metapleura, we see no sure ground for inferring its origin, either from the loss of one, or from the fusion of both of a primitive pair of organs. Yet the indications are that the median fin is not so much single by fusion as by loss of its fellow.

As illustrating the ease with which paired lateral structures may pass to a median ventral position and *vice versa*, this case is of interest with reference to the origin of appendages of fishes from lateral fins and their connection with a median fin.

The disappearing metapleura on the sides of the pre-oral chamber are intimately associated with the pre-oral hood. This structure is much more extensive than in the common Amphioxus, forming a deep shovel-shaped or inflated membrane suspended from the posterior and lateral margins of the pre-oral depression; when expanded it appears as in Fig. 6.

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From a side view (Fig. 1, 2, 3, 4) it extends forward ventrally over the pre-oral chamber like a hood, with cirri like those of other lancelets. These cirri, however, are perfectly smooth as shown in Fig. 19 and do not present the denticulate outlines due to the special sense organs found on the cirri of other Acraniata. The number of cirri is 15 to 21, the small lateral, anterior ones being involuted dorsally with the membrane and not seen from a ventral view (Fig. 6). There may be a median cirrus and an equal number of paired cirri on each side, as seen in the view of the macerated skeleton (Fig. 24). Each cirrus is supported by a special cartilage-like rod springing from a common curved and jointed basal arch, as in the common lancelet. Each rod, it will be observed, springs from the distal end of one of the basal pieces composing the common basis for all the rods. The hood differs from that of the European form, and from that of the Florida form also, in having the membrane continued up over nearly the whole length of the cirri, leaving but their tips free. Moreover the median cirrus is so long, and the most lateral so short that there is no reason to doubt that the latter are the youngest as has been shown to be the case in Amphioxus (**27**) although before the embryological facts were known the examination of the adults had led Lankester (**23**) to the opposite conclusion.

If we were justified in regarding the lateral edges of the pre-oral chamber as continuations of the metapleura, it is tempting to suppose that the hood membrane, lying between those continued metapleura and posteriorly continuous with the floor of the atrium (Fig. 6) may be homologous with the sub-atrial ridges, may have been originally an anterior continuation of those parts of the metapleura which now unite posteriorly to form the floor of the atrium. Such a view may be supported by the fact that in amphioxus, as Willey has discovered (**27**), the oral hood arises from two beginnings one right and one left: while other facts that militate against it may be explained as secondary acquisitions connected with the remarkable asymmetry of the oral region of the Acraniata.

In addition to the above peculiar features of the reproductive organs and fins, the Bahama lancelet presents some few minor anatomical features which may now be described in comparison with the similar structures in the European forms.

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The true mouth is at the centre of a transverse velum provided with a strong sphincter muscle and a series of oral tentacles that are not, apparently, quite as numerous as in the European lancelet though having the same arrangement.

The capacious pre-oral chamber bears upon its side wall the so-called "Räderorgan," consisting of three large ridges of specially thickened, ciliated epithelium, passing forward on each lateral wall (Figs. 1, 2) to end in rounded lobes in the posterior half of the chamber above the basal part of the pre-oral hood. These ridges might be regarded as one continuous ridge folded back and forth into three chief loops with smaller lobes interpolated between them at the posterior end. All the ridges continue posteriorly as a ciliated area leading to the velum and out on it towards its central aperture, the mouth. An important part of the structure is a special ridge found only upon the right side, high up towards the notochord, so that it appears median from a side view (Fig. 1). In sections anterior to the above paired ridges (Fig. 13) this right, azygos ridge continues to end only at the extreme anterior angle of the pre-oral chamber. Here it is associated with the conspicuous pre-oral pit found on the right side in the position shown in Fig. 12, but continues as a special ciliated ridge even anterior to the pit (Fig. 18) as well as posteriorly to join the other ridges at the velum.

The pit has the form of a glandular tube, somewhat bent, opening out into the pre-oral chamber in the azygos ridge and histologically divided into two regions. The terminal part is ciliated, the internal closed end has an epithelium of what appear to be gland cells casting out a coagulable substance into the lumen. As already stated the two regions of the organ behave differently when the animal is fed with carmine.

That the pit has either an excretory function or, more probably, may serve to secrete slime to aid in entangling food particles to be swept back to the mouth, is an idea suggested by its action towards carmine and strengthened by its anatomical relations to a large blood vessel or space. This vessel passes along close beneath the azygos ridge for its whole length (Figs. 12, 13) and is the "aortic arch" of Langerhans (**28**) as figured by Ralph (**29**) and described by Lankester (**23**) and recently called a "glomus" by van

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Wijhe (**32**), who regards it as of excretory function. It seems to be much less complex than in the European form; though it presents some branches anteriorly it is not to be regarded as a glomus here. Posteriorly it passes between the velum and the lateral muscles but could not be traced into connection with other vessels. Anteriorly it passes between the walls of the pit and the azygos ridge, close to each end bends up dorsally between the tip of the pit and the end of the ridge. Here it immediately unites, at the anterior end of the pre-oral chamber, with a large branch of the left aorta, forming thus a median aortic trunk that runs forward a short distance, close beneath the notochord, bifurcates, and ends in the ventral sheath of the notochord. This left aorta is notably smaller than the "right arch" (Figs. 12, 13) and branches just before the anterior end of the pre-oral chamber is reached, sending the larger branch to fuse with the "right arch," as above stated, and the smaller branch forward a short distance to branch in turn and, perhaps, to connect with the large median lymph space beginning here and lying under the notochord as seen in Fig. 10.

Of the two aortic trunks found in the pharyngeal region it is only the left that connects, as above, with the "right arch." The right aorta seems to disappear in the velum.

No attempt has been made to trace out the rest of the vascular system though the chief aortic trunk, the vessels of the branchiae, and the numerous vessels on the posterior intestine and caecum seem, in sections, to be much as described for the common Amphioxus.

The branchial apparatus is like that of the known forms; but as seen in Figure 2 the first gill-slit may be small and only partly divided by a tongue bar, while in the European species van Wijhe (**32**) finds that the first gill slit has no tongue bar. In macerations of the branchial skeleton we find the usual primary bars and tongue bars represented by rods that are double in the former, divaricating at the lower end and deeply cleft lengthwise, but apparently single in the latter, though marked longitudinally by cavities and grooves.

Primary rods and tongue bar rods unite dorsally, so that they form a series of three-tined forks.

The usual number of horizontal connecting bars pass across the tongue bar from one primary to the next. Dorsally the processes

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of the primary rods called by Spengel (**30**) the "Bügelstücke" are very large and prominent; ventrally, however, the endostylar plates could not be demonstrated by maceration or by section. Such sections give the usual marked difference between the primary and secondary bars, but on account of their small size would make difficult any attempt to trace the exact course of the blood vessels as determined by Lankester (**23**) or by Spengel (**30**).

In the digestive tract of this Bahama amphioxus there is a marked zone of small, crowded cells that take up stain readily and form a dark band posterior to the stomach region as indicated in Fig. 5. This is found in the Florida specimens of *Branchiostoma caribæum*, but appears not to have been noticed in the European Amphioxus.

The atrial chamber has the usual longitudinal, ventral ridges that have been regarded as of excretory function, but a long search for the nephridial tubules found by Boveri (**31**) was without success, probably owing to the small size of the specimens and the distortion due to preservation. The live specimens were not examined for this purpose. Surface preparations of preserved specimens of the Florida *B. caribæum* showed nephridia like those obtained by Boveri by this method, but none were made out in the small Bahama animals. This atrial chamber differs from that of European forms in the extent of its posterior portion. Thus while Lankester (**23**) finds a single pouch running back of the atriopore "as far back as the anus" there are here two short pouches, the right longer than the left, that seem to have pushed themselves back on either side of the atrial outlet and then, very soon, stopped growing. Both are shown in Figure 15, which is posterior to the base of the atrioporal spout, though showing its tip; further back Figure 21 shows no representative of the atrium.

In the Florida *B. caribæum* there is a large post-atrioporal pouch on the right, but it does not reach as far as to the anus.

In the youngest individuals taken, the floor of the atrium is but imperfectly formed, the two sub-atrial ridges being incompletely fused on the median, ventral line.

Here there is an interesting segmented arrangement of the muscle fibers such as has not been noticed before, I believe. The transverse muscle of the atrial floor (Fig. 14) begins as a series of

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fibers in each sub-atrial ridge before they unite; there is thus a double series of transverse fibers that subsequently unite with one another across the middle line. In each half of the series, on the right and on the left, the fibers have the peculiar grouping seen in Figure 16. There are three or four groups for each myotome, each group being due to a special centre of growth about which the fibers are arranged in pairs and become gradually longer as they adjoin the other groups. Eventually all the fibers seem to become of equal length so that this segmentation is obliterated.

The agreement of the groups upon opposite sides of the median line is also noticeable as a marked case of bilateral symmetry in the arrangement of individual cells upon disconnected, opposite sides of the body.

No thorough examination of the nervous system was attempted, but it seems to present no marked difference from the state of things found in the common amphioxus. There are dorsal and ventral nerves and amongst the former a large one, seen in Fig. 10, that arises from the posterior part of the brain, branches freely and is distributed to the anterior part of the median fin where there seem to be terminal organs in the living specimen. Two slender nerves continued from the anterior end of the brain, along the notochord are sometimes seen to have a hollowed out base which may be an indication of what Ayres (**34**) has described as optic diverticula or lobes in the common lancelet, which, however, seem here to be rather the results of distortion in preparation than normal conditions.

The ventricle of the brain has in longitudinal median section very much the form and proportion represented by Küpffer (**33**), Figs. 21–22, but the posterior ventral diverticulum could not be recognized as a cavity though indicated by a non-nucleated, clearer region.

In transverse sections we have successively the appearances seen in Figs. 7, 8, 9, which represent the ventricle at its anterior, middle and posterior portions with the relative size of lumen and wall.

The pigment spot at the anterior end of the brain presents nothing to suggest that it is in any sense an eye. It is not placed exactly as in the above figures of Küpffer but forms a terminal cap-shaped deposit in the conical tip of the brain, is a collection of pigment in the conical tip of the ventricle wall and is thus cut as a ring of

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pigment in a few, very thin, sections immediately posterior to the extreme tip of the ventricle, Fig. 7.

As far as could be observed in surface views of both living and preserved specimens and in transverse and longitudinal sections of both young and adults no anterior neuropore or "nasal pit" exists, nor any such connection of the anterior part of the brain with the epidermis.

The pigment spots of the neural tube are very conspicuous in life and in sections as large cap-shaped masses about individual ganglion cells of the ventral part of the cord near the base of the median fissure or canal.

The spots begin a short distance posterior to the brain and soon become more crowded to form a series, on each side, that ends posteriorly before the caudal process is reached. There is throughout a disposition to aggregation in linear groups suggesting a metameric arrangement, but the short lines of pigment spots are not actually arranged with reference to the myotomes.

This pigment is very resistant, as attempts to remove it in Grenacher's depigmenting liquid were not successful after many days' immersion.

In summarizing the above account of the Bahama amphioxus, we find that its peculiar structures, asymmetry of the reproductive organs and great extension of the tail fin, do not throw light upon the morphology of the acraniates, but are such characters as may be explained as secondary departures from a type more like the common European form. Thus the lack of the left gonads is no doubt a loss of what was at one time present. The extension of the tail is also a secondary feature; for we will see later that the actual number of myotomes has not been decreased, on the contrary there are 66 here as compared with 61 in the European lancelet, so that we have no ground for supposing that the caudal process was once accompanied by myotomes. It is rather a posterior outgrowth of use in swimming, a true caudal process. Yet the presence of the neural tube in this non-muscular region does suggest the former existence of muscles; on the other hand the extension of the brain beyond the anterior myotomes (Fig. 10) furnishes evidence as to the possibility of nerve tube and notochord extending out together into new terminal structures.

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Both the character of the fins and the diminution in bulk of the gonads, by loss of one series, may be explained on the assumption that they favor more active locomotion outside the sand. We have evidence that this species is, to some extent, free-swimming in habit.

In order to determine what taxonomic value should be attached to the anatomical features of this Bahama lancelet it will be necessary to review our knowledge of known Acraniates as far as it concerns the systematic work upon members of the group and the closely associated facts of geographical distribution.

Historical.

As is well known the first recorded account of Amphioxus was that of Pallas (1), who in 1774 described a preserved specimen that came from the Cornish coast. Though the fish-like characters of this undoubted Amphioxus were recognized by Pallas ("quodque prima facie referet Piscem Leptocephalum Gronovii") it was described and figured as a mollusc, *Limax lanceolatus*.

This error was corrected by Costa (2) who more than half a century later re-discovered the animal, on the shores of the Mediterranean, and misled by the gill-like appearance of the pre-oral cirri, named it *Branchiostoma lubricum*. There seems to be no doubt that this account was published in 1834, though in a paper so difficult of access that it is chiefly the author's statements made in the two complete, illustrated descriptions (3) of 1838 and 1843 that we rely on in selecting this name *Branchiostoma* as preferable to the one commonly used, Amphioxus.

This latter was given in 1836 by Yarrell (4), who included in his "British Fishes" an illustrated account of a single specimen taken by Mr. Couch, at Polperro on the Mediterranean, where it happened to be lying with its tail protruding from under a stone.

Not knowing the work of Costa, though acquainted with the description of Pallas, Yarrell invented the name *Amphioxus lanceolatus* under which the animal soon became an object of much interest and subject of many anatomical investigations.

The older generic term was used again by J. E. Gray (5), in description of a new species *B. Belcheri* from Borneo. This was obtained at the mouth of the river Lundu by Capt. Belcher of H.

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M. S. "Samarang" and "as what appears to be a new species of Lancelet," is described in a rather vague and unsatisfactory way from a single specimen. Another was sent to Dr. Clarke, R. N., for anatomical study, which appears never to have been made public.

Moreover the author compared this new form with specimens from Cornwall and from the Mediterranean and concluded they were all three different species. This opinion was maintained again in the subsequent Catalogue of the British Museum (**6**) where Gray calls the Naples form *B. lubricum*, that from Borneo *B. Belcheri* and those from Polperro and S. W. England as well *B. lanceolatum*.

In the following year Sundewall (**7**) added to the knowledge of the group by his description of the specimens in the Museum of Stockholm. Amongst these he distinguished a new species *A. elongatus*, sent by Capt. Wärngren from Chinchaõarna, Peru, from *A. lanceolatus*, from the German Ocean as well as from the coast of Cornwall. He included Gray's Borneo species in the genus Amphioxus, as *A. Belcheri*.

Soon afterwards (**8**) he recast these descriptions and, for the first time, introduced the counting of the myotomes, or the muscle-segments, as a means of distinguishing species of Acraniata, counting not only the entire number of segments but also the number anterior to the atriopore, between that and the anus and posterior to the anus, thus defining the relative positions of these important structures.

Branchiostoma lanceolatum (as he now calls it) has $36 + 14 + 11 = 61$ myotomes in specimens from Northern Europe. *B. elongatum*, from Peru, $49 + 18 + 12 = 79$, while a new form, *B. caribœum*, found at St. Thomas, at Rio de Janeiro and at the mouth of the Plata has $37 + 14 + 9 = 60$.

The four forms thus far described were not, however, regarded, generally, as specifically distinct. Thus Guenther (**9**) in 1870 in his catalogue of the specimens in the British Museum included all known forms under the single species *B. lanceolatum*.

However, in 1876 some new specimens were brought from Moreton Bay, Peale Sound, Northeast Australia, by Captain Schleinitz of S. H. S. Gazelle and described by Peters (**11**) as generically different from all known forms. As far as could be

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determined from alcoholic specimens, the anatomy of this new *Epigonichthys cultellus* was the same as in Branchiostoma. No enumeration of the muscle segments was made, but the character of fins and the apparent median position of the anus were regarded as of generic value.

Subsequently Guenther (**10**), in the only complete comparison of all known forms of Amphioxus that has thus far been attempted, was able to examine specimens of this new genus brought from Thursday Island, Australia, by Dr. Copinger. Comparing these with other Acraniata he referred them back to the genus Branchiostoma.

Moreover, Guenther now regarded the others as specifically distinct, so that in this list of the Acraniates in the British Museum we find the above form appearing as *B. cultellum* then *B. lanceolatum* from Europe and from the Atlantic Coast of North America, *B. elongatum* from Peru (on the authority of Sundevall), *B. Belcheri* of Gray, represented also by new specimens brought by Dr. Copinger from Prince of Wales Island, Torres Straits, and finally *B. caribæum*, which Van Beneden got in large numbers at the Bay of Botafago and which furnished the material for Moreau's study of the neural cord (possibly this is the same as *Amphioxus Mülleri* of Kröyer's MSS.).

In addition to these previously known forms this revision adds a new one, *B. bassanum*, from Bass Straits; this was formerly regarded by Guenther as *B. lanceolatum*. Of these six species five are represented by specimens in the British Museum.

About this same date, 1884 or '85, as my friend Dr. S. Watase informs me, Professor Matsubara of the Medical Department of Tokio University read to the Zoölogical Society of Tokio an account of an Amphioxus found at the island of Kinshin, in the western part of Japan.

Whether this is a new species or not remains to be seen.

Earlier than that, in the winter of 1877–8, as appears from a brief note by Professor Brooks (**19**), an assistant in the Smithsonian Institution found some sort of Amphioxus at the Bermuda Islands.

The knowledge of the distribution of Sundevall's *Branchiostoma caribæum* was extended in 1891 by the discovery of small miniature specimens swimming at the mouth of Kingston Harbor,

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Jamaica. These specimens were taken by the Johns Hopkins Marine Laboratory and merely mentioned as Amphioxus (**21**), but subsequent examination has proved their identity with the forms previously known from St. Thomas, South America and the Southern United States.

As early as 1887 the same Laboratory while at Nassau, N. P., obtained miniature swimming forms in the harbor. These were taken again by us in July of 1892 at the same place, and proved to be immature specimens of what we will call *Asymmetron lucayanum*, so that we may regard this form as probably occurring all through the Bahama Islands.

Meanwhile there had appeared brief mention of the occurrence of Acraniates at various points upon the coast of North America.

Thus, in an account of the Zoölogy of California (**12**) published in 1868, J. G. Cooper mentions the occurrence of *Branchiostoma—?* found in the sand at San Diego, California, and gives a popularized characterization that leaves no doubt as to the acraniate character of the creature. From the notice of Gill (**13**) it appears that these Californian lancelets were dredged at a depth of ten fathoms, and that only three specimens were found. One of these is now in the collection of the Smithsonian Institution, labelled *B. californiensis*. As this and many others from the same locality have an arrangement of myotomes unlike that of described species, we may conclude that it is specifically distinct though it has been referred to as *B. lanceolatum*, by Jordan and Gilbert (**14**), who had, however, not seen the specimens, and regarded (**15**) all American forms as not specifically distinct from the European, as far as had been proven.

On the Atlantic coast Coues and Yarrow (**16**) dredged a dozen or more specimens on Bird Shoal, Beaufort, N. C. They identified these as *Branchiostoma caribæum*. This discovery was, however, spoken of by Jordan and Gilbert in their list of Beaufort Fishes (**17**) under the name *Amphioxus caribæus*, though later, as above mentioned, all American forms were regarded as undistinguished from the European.

At the same place Amphioxus was collected by members of the Johns Hopkins University in 1880–1–2–4–5.

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They also found adult and larval forms in 1878, by dredging in water 12 to 15 feet deep near Ft. Wool, at the mouth of the Chesapeake.

Larval forms were taken at the surface in July and August. These were figured and described by Rice(18) as *Amphioxus lanceolatus*.

From his statement it appears that one specimen had been previously found upon the Eastern Shore of Virginia by Mr. P. R. Uhler, and that it was then known that Amphioxus had previously been found in Florida as well.

Yet the first published notice of the occurrence of Acraniata in Florida seems to be that of A. A. Wright (20) who obtained large numbers at Port Tampa in March 1890. Its previous discovery in Florida rests upon the above statement of Rice and upon the presence of specimens in the Smithsonian Museum.

Acting upon information kindly furnished by Prof. Wright, members of the Johns Hopkins Laboratory obtained large numbers of adult and larval *Branchiostoma caribæum* at Port Tampa in June 1892, which enables us to extend the distribution of this species as will be seen in the summary of geographical distribution.

From the above review it will be seen that there is but one genus of Acraniates, Branchiostoma, and that the various species from different parts of the world have been distinguished by the only marked differential characters seen in alcoholic specimens; the number and arrangement of the myotomes. Lankester (23) leaves the question as to the fixity of these numerical characters, an open one. So great is the difficulty of accurate counting, where the terminal myotomes may be very small and vaguely demarked in preserved specimens, that we do not know the limits of variation in any one species. Successive counts of one specimen of *B. caribæum* have given me such numbers as 35. 14. 9; 35. 13. 10 and 35. 15. 9; for the anal and atrial openings are also not precisely localizable in preserved specimens.

In the first appendix to this paper I have given all the enumerations of myotomes that could be found in the systematic literature and in addition a considerable number made upon specimens in the Smithsonian Institution and elsewhere. From a consideration of

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these, there is no doubt, I think, that such enumerations may be relied upon as giving safe specific criteria though they should not by any means be the only ones, if live specimens can be observed. This in spite of the great difficulty in making accurate counts and allowing for the probable, slight variations within the species. Until the various forms can be monographed from a study of living specimens such characters will be most useful.[1]

Admitting that the number and arrangement of the myotomes furnish criteria of specific value in the group Acraniata, we must concede that such peculiarities of the fins and reproductive organs as we have found in the Bahama Amphioxus are of at least generic value and justify the establishment of a new genus to be characterized as follows:

Asymmetron g. n.[2]

Gonads present only upon the right side.

Ventral fin with no fin rays or successive fin-ray chambers. A long caudal process.

The only known species, *Asymmetron lucayanum*,[3] may be characterized as follows:

Right metapleuron continuous with the median ventral fin, which passes to the right of the anus. Pre-oral hood extensive, the cirri united by the membrane throughout the greater part of their length and smooth, without sensory papillæ. Gonads on the right 29, extending from the 15th to the 43d myotomes inclusive. Myotome formula 44 . 9 . 13 = 66. Length 13 mm. "Olfactory pit" apparently absent; nephridia and brown canals not demonstrated.

Adults and young swimming at surface in the evening in June and July at Bemini and Nassau, Bahamas. Also taken buried in the calcareous sand.

With this addition we may form the following summary of our knowledge of the classification of the group Acraniata.

[1] For the aid of any who may wish to know of the existence of preserved specimens, a list of those in the Smithsonian Museum is given in Appendix II.

[2] *ἀ-σύμμετρος, ον* = wanting symmetry.

[3] From the Lucayas; the islands discovered by Columbus in 1492, now the Bahamas.

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ACRANIATA.

Genus BRANCHIOSTOMA. Gonads present on both sides; ventral fin with fin-rays; no caudal process.

1. *B. lanceolatum:* formula 36 . 14 . 12 = 61, length 37 mm. Distribution, Scandinavia, England, Mediterranean, Chesapeake Bay? Fiji Islands?
2. *B. caribæum:* formula 35 . 14 . 9 = 58, length 43 mm. Distribution, Mouth of La Plata, Brazil, St. Thomas, Jamaica, Tampa Bay and Gulf of Mexico, Beaufort, N. C.
3. *B. cultellum:*[1] formula 32 . 11 . 10 = 52, length 23 mm. Distribution, Moreton Bay and Thursday Island, Australia.
4. *B. bassanum:* formula 44 . 14 . 17 = 75, length ———. Distribution, Bass Straits, Australia.
5. *B. belcheri:* formula 37 . 14 . 14 = 65, length 65 mm. Distribution, Borneo and Torres Straits, Australia.
6. *B. elongatum:* formula 49 . 18 . 12 = 79, length 60 mm. Distribution, Chinchaöarna, Peru.
7. *B. Californiense:* formula 44 . 16 . 9 = 68, length 70 mm. Distribution, San Diego, California.

Genus ASYMMETRON; Gonads present only on right; ventral fin with no fin-rays; a long caudal process.

8. *A. lucayanum:* formula 44 . 9 . 13 = 66, length 13 mm. Distribution, Bimini and Nassau Harbor, Bahamas.

In addition to the species enumerated above there are records of lancelets found in other regions that extend the area of geographical distribution and may modify our conception of the number of specific forms when all these specimens shall be re-studied. At present, then, the geographical distribution of the Acraniata may be summarized as follows:

EUROPE. Scandinavian, British, and Italian Coasts: (1) *B. lanceolatum.*

ASIA. Borneo: (1) *B. Belcheri.* Japan: (2) —— ——? (3) Ceylon; —— ——?

[1] Mr. Arthur Willey of Columbia College informs me that he finds only right gonads in this species; so I judge it should be referred to the genus Asymmetron, in a future revision of the Acraniata.

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AUSTRALASIA. North Australia: (1) *B. cultellum* and *B. Belcheri*. East Australia: (1) *B. cultellum*. South Australia: (3) *B. bassanum*. Fiji Islands: (4) *B. lanceolatum?* (Rohon (22) quoting C. Hesse of Hamburg.)

AMERICA. Pacific Coast: (1) *B. elongatum*, Peru; and (2) *B. Californiense*, California. Atlantic Coast: (3) *B. caribæum*, South America and Southern United States; and (5) *B. lanceolatum?* mouth of Chesapeake Bay. West Indies: (3) *B. caribæum*, Antilles; and (4) *A. lucayanum*, Bahamas. Bermudas: —— ——?

APPENDIX I.

(Formulas marked * were determined more accurately than others.)

Branchiostoma lanceolatum.

				mm.				
36.	14.	11.	61.	23–44.	Northern Europe:	Sundevall (8).		
35.	12.	12.	59.		Polperro :	Günther (10).		
34.	13.	13.	60.		Naples	"		
35.	12.	13.	60.		"	"		
35.	15.	10.	61.		Naples :	Lankester (23).		
35.	14.	13.	62.		"	"		
35.	14.	12.	61.		"	"		
35.	16.	10.	61.		"	"		
36.	15.	11.	62.		"	"		
36.	13.	12.	61.	37.	Stavanger, Norway (Smith. Mus.)			E.A.A.
37.	13.	12.	62.	27.	"	"	"	"
35.	13.	10.	58.	27.	"	"	"	"
35.	12.	13.	60.	27.	"	"	"	"
36.	13.	12.	61.	37.	"	"	"	"
36.	15.	10.	61.	52.	Sicily: J. H. U. Mus.			"
35.	14.	11.	60.	52.	"	"		"
35.	14.	11.	60.	35.	Naples: (Bryn Mawr Mus.)			"
36.	14.	11.	61.	38.	"	"		"
36.	13.	13.	62.	37.	"	"		"
37.	14.	11.	62.		"	"		"
36.	13.	11.	60.	36.	"	"		"
33.	14.	12.	59.	42.	"	"		"
35.	13.	13.	61.	40.	"	"		"
36.	14.	12.	62.	42.	"	"		"
35.	14.	12.	61.	42.	"	"		"
35.	14.	11.	60.	45.	"	"		"
36.	14.	12.	62.	44.	"	"		"
36.	14.	11.	61.	40.	"	"		"
36.	13.	11.	61.	43.	"	"		"
36.	14.	12.	62.	42.	"	"		"
36.	13.	12.	61.	44.	"	"		"
35.	14.	13.	62.	37.	Italy?	"		"
35.	14.	12.	59.	34.	"	"		"

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				mm.		
36.	14.	13.	63.	37.	Italy? (Bryn Mawr Mus.)	E.A.A.
36.	14.	12.	62.	36.	" "	"
36.	14.	13.	63.	36.	" "	"
35.	13.	12.	60.	36.	" "	"
36.	13.	12.	61.	37.	Naples: "	"
35.	14.	11.	60.	37.	" "	"
37.	13.	13.	63.	39.	" "	"
37.	13.	12.	62.	37.	" "	"
38.	13.	13.	64.	36.	" "	"
37.	14.	12.	63.	43.	" "	"
35.	13.	12.	60.	40.	" "	"
36.	13.	13.	62.	38.	" "	"
36.	13.	12.	61.	50.	" "	"
35.	14.	11.	60.	34.	Naples? "	"
36.	13.	15.	64.	53.	Chesapeake, Ft. Monroe (J. H. U. Mus.) E.A.A.	
36.	13.	13.*	62.*	51.	Chesapeake, Ft. Monroe (J. H. U. Mus.) E.A.A.	
36.	13.	12.	61.	28.	Chesapeake, ———— (J. H. U. Mus.) E.A.A.	
37.	13.	11.	61.	34.	Chesapeake, Willoughby Sandspit. E.A.A.	
36.	16.	7.	59.	20.	Chesapeake: 4 m. East Sewell's Point (Smithsonian Mus.) E.A.A.	
33.	14.?	12.?	59.	40.	Ceylon (Smith. Mus.) E.A.A.	
35.6	13.6	11.8	61.	38.4	Average.	
36.	14.	12.	61.	37.	Most frequent.	

Branchiostoma caribæum.

				mm.		
37.	14.	9.	60.	21–51.	St. Thomas and Rio de Janeiro: Sundevall (8).	
37.	14.	9.	60.			
37.	13.	9.	59.		Botafago, Janeiro and Mouth of La Plata: Guenther (10).	
36.	13.	9.	58.	42.	Florida, St. Martin's Reef (Smith. Mus.) E.A.A.	
34.	15.	9.	58.	32.	Gulf of Mexico (Smith. Mus.) E.A.A.	
36.	15.	8.	59.	31.	" " "	
36.	14.	9.	59.	45.	Florida, Port Tampa (J. H. U. Mus.) E.A.A.	
35.	14.	9.	58.		Florida, Port Tampa (J. H. U. Mus.) E.A.A.	
36.	15.	8.	59.	43.	Florida, Port Tampa (J. H. U. Mus.) E.A.A.	
35.	15.	10.	60.	45.	Florida, Port Tampa (J. H. U. Mus.) E.A.A.	
36.	15.	9.	60.	38.	23 r–22 l gonads (J. H. U. Mus.) E.A.A.	
35.	14.	8.	57.	30.	" "	"
35.	14.	9.	58.	46.	26 r–25 l gonads "	"
36.	14.	9.	59.	43.	26 r–26 l " "	"
35.	14.	9.	58.	40.	27 r–24 l " "	"
35.	14.	9.	58.	43.	26 r–26 l " "	"
35.	14.	9.	58.	30.	24 r–23 l " "	"

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32.*	15.	10.	57.		Florida, Port Tampa (J. H. U. Mus.) Knower.
32.*	15.	10.	57.		Florida, Port Tampa (J. H. U. Mus.) Knower.
35.	17.	7.	59.		Florida, Port Tampa (J. H. U. Mus.) Knower.
33.	12.	9.	54.	8.5	Jamaica, Kingston (J. H. U.) E.A.A.
27.*	12.	9.	48.	8.†	" " " "
36.	12.	9.	57.	13.	" " " "
34.8	14.	8.9	57.8	33.6	Average.
35.	14.	9.	58.	43.	Most frequent.

Branchiostoma Belcheri.

37.	14.	13.	64.	Borneo : Guenther (10).
37.	14.	14.	65.	Prince of Wales Islands: Guenther (10).
37.	14.	13.5	64.5	

Branchiostoma cultellum.

				mm.	
32.	10.	10.	52.		Thursday Island: Guenther (10).
31.	11.	10.	52.	13–23.	Moreton Bay: Peters (11).
31.5	10.5	10.	52.		

Branchiostoma Californiense.

44.	16.	9.	69.	57.	California, San Diego (Cooper's type, Smith. Mus.) E.A.A.
44.	13.	9.	66.	70.	California, San Diego (Smith. Mus.) E.A.A.
45.	15.	8.	68.	70.	California, San Diego (Smith. Mus.) E.A.A.
44.	16.	9.	69.	68.	California, San Diego (Smith. Mus.) E.A.A.
43.*	16.	9.	68.+	70.	California, San Diego (Smith. Mus.) E.A.A.
42.	16.	8.	64.	65.	California, San Diego (Smith. Mus.) E.A.A.
43.	16.	8.	67.	57.	California, San Diego (Smith. Mus.) E.A.A.
43.8	15.4	8.5	67.3	65.3	Average.
44.	16.	9.	68.	70.	Most frequent.

Branchiostoma bassanum.

44.	13.	18.	75.	Bass Straits: Günther (10).
43.	15.	17.	75.	" " "
45.	14.	17.	76.	" " "
44.	14.	17.3	75.3	Average.

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Branchiostoma elongatum.

49.	18.	12.	79.	39–60.	Peru, Chinchaôarna: Sundevall (7 and 8)

Asymmetron lucayanum.

				mm.	
43.	8.	14.	65.	12.5	Bahamas, Bemini (J. H. U. Mus.) E.A.A.
45.	9.	12.	66.	12.5	" " " "
44.	9.	11.	64.	12.	" " " "
44.	9.	13.	66.	12.5	" " " "
44.	9.	13.	66.	10.	" " " "
43.	9.	12.	64.	11.	" " " "
42.	8.	12.	62.	10.	" " " "
45.	8.	13.	66.	13.	" " " "
43.	9.	14.	66.	13.	" " " "
42.	9.	13.	65.	9.	" " " "
44.	9.	12.	65.		" " " "
44.	8.	14.	66.	12.5	" " " "
44.	8.	13.	65.	12.	" " " "
43.	9.	13.	65.	7.5	" " " "
46.	9.	13.	68.	12.5	" " " "
44.	9.	12.	65.	13.	" " " "
45.	8.	13.	66.	14.	" " " "
43.	9.	12.	64.	14.5	" " " "
44.	9.	13.	66.	14.5	" " " "
43.	9.	12.	64.	14.	" " " "
44.	9.	13.	65.	16.	dug. " " " "
46.	9.	13.	68.	14.	" " " "
43.8	8.7	12.7	65.3	12.4	Average.
44.	9.	13.	66.	13.	Most frequent.

APPENDIX II.

Catalogue of Specimens of Amphioxus in the Smithsonian Museum, with numbers and names as there attached.

(1). 39380. 242. *Amphioxus lanceolatus.* One small specimen from St. Martin's Reef, Florida. Jeff. F. Moser.

(2). 43555–8. *Branchiostoma caribœum.* 18 spec. obtained by U. S. F. C. Schooner Grampus, dredging at stations 5064, 5068, 5066, 5111, in 1889.

(3). 27763. *Amphioxus lanceolatus.* About 50 small spec. obtained by Dr. Bessels 4 miles east of Sewell's Point, Virginia (Chesapeake).

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(4). 4372. 244. *Branchiostoma lanceolatum.* 21 very large specimens from San Diego, California. L. C. Bragg.

(5). 39646. 242. *Branchiostoma lanceolatum.* 2 spec. (one macerated) from San Diego, California. H. Hembhill.

(6). 8407. 244. *Branchiostoma Californiensis.* One specimen from San Diego, California. Cooper.

(7). 23020. 242. *Branchiostoma lanceolatum.* 3 spec. from Stavanger, Norway. R. Collett.

(8). 22083. 242. *Branchiostoma lanceolatum.* 2 spec. from Stavanger, Norway. R. Collett.

(9). 34003. 244. *Amphioxus* . Five soft specimens from Ceylon. Dr. Francis Day.

REFERENCES.

(1). Pallas: Spicil Zoöl. X, p. 19, Pl. I, Fig 11.

(2). Costa: Cenni Zoölogici, Napoli, 1834, p. 49.

(3). Costa: Fauna del Regno di Napoli, Fas. 21, 1838. Storia e Natomia del Branchiostoma lubrico. Frammenti Anatomia Comparata, Fas. I, Napoli, 1843.

(4). Yarrell: British Fishes, 1836, p. 462.

(5). Gray: Proc. Zoöl. Soc., 1847, pp. 35-36.

(6). Gray: Cat. Brit. Mus., Fish, Vol. 7, p. 150.

(7). Sundewall: Öfervs. Vet. Akad. Förh., Vol. IX, 1852, pp. 147–148.

(8). Sundevall: Öfervs. Vet. Akad. Förh., Vol. X, 1853, pp. 12–13.

(9). Guenther: Catalogue of Fishes, Brit. Museum, 1870, Vol. VIII, p. 513.

(10). Guenther: Report Zoöl. Collec. H. M. S. Alert, Brit. Mus., 1884, pp.

(11). Peters: Monatsberichte K. Preus. Akad. Wiss., Berlin, 1876, pp. 322–327, Pl. I, 5 Figs.

(12). Cooper: Nat. Wealth of California. Cronise, 1868, p. 498.

(13). Gill: Notes on Leptocardians. Proc. Nat. Mus., V, 1882.

(14). Jordan and Gilbert: Fish of Pac. Coast, U. S. Proc. Nat. Mus., Vol. IV, 1881, p. 29.

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(15). Jordan and Gilbert: Synopsis Fishes of N. America. Bull. U. S. Nat. Mus., 1882, p. 867.
(16). Coues and Yarrow: Nat. Hist. of Fort Macon, N. C. Proc. Phil. Acad. Sci., 1877, p. 218.
(17). Jordan and Gilbert: Fishes of Beaufort Harbor. Proc. Nat. Mus., Vol. I, 1878, p. 388.
(18). H. J. Rice: Amphioxus. American Naturalist, Vol. XIV, 1880, pp. 1–19, Pl. I–II.
(19). Brooks: Ann. Report Pres. J. H. Univ., 1878, p. 49.
(20). Wright: Amphioxus at Tampa. Amer. Naturalist, Vol. 24^2, 1890, p. 1085.
(21). Andrews: Fauna of Jamaica. J. H. U. Circulars, April, 1892, p. 75.
(22). Rohon: Amphioxus lanceolatus. Denkschrift Acad. Wiss. Wien. Math.-Nat. Cl. 45, 1882.
(23). Lankester: Amphioxus lanceolatus. Q. J. Mic. Sci., 29, 1889, p. 370.
(24). Weiss: Excretory Tubules in Amphioxus. Q. J. Mic. Sci., 31, 1890, pp. 489–496, Pl. 34–35.
(25). Boveri: Bildungsstätte der Geschlechtsdrüsen beim Amphioxus. Anat. Anz., VII, No. 6, March, 1892.
(26). Lankester and Willey: Atrial Chambers of Amphioxus. Q. J. Mic. Sci., 31, 1890.
(27). Willey: Larval Development of Amphioxus. Q. J. Mic. Sci., 32, 1891.
(28). Langerhans: Anatomie der Amphioxus. Archiv. f. Mik. Anat., XII, 1876.
(29). Rolph: Ban des Amphioxus. Morphologisches Jarb., 2, 1876.
(30). Spengel: Kiemen des Amphioxus. Zoöl. Jarb., IV, 1891.
(31). Boveri: Nierencänalchen des Amphioxus. Zoöl. Jarb., V, 1892.
(32). van Wijhe: Amphioxus. Anat. Anz., VII, Feb., 1893.
(33). Küpffer: Kopfe der Kranioten. 1893.
(34). Ayres: Cephalogenesis. Journal Morph., IV, 1891.

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EXPLANATION OF PLATES.

All the figures are reduced from camera lucida drawings of *Asymmetron lucayanum.*

PLATE XIII.

Figure 1. Right side of an adult having 45 . 8 . 14 = 67 myotomes; reduced to $\frac{1}{8}$ the diameter of the original drawing from the living specimen.

Figure 2. Left side of the same specimen; from a drawing made a day later than the above.

Figure 3. Left side of a preserved immature specimen having 43 . 8 . 15 = 66 myotomes and 27 gill-slits. The small ventral caecum is shown at *c*.

Figure 4. Left side of an opaque, preserved specimen 14 mm. long, having 44 . 8 . 13 = 63 myotomes, gill-slits from the 10th to 35th myotomes and 28 gonads extending from the 16th to the 43d myotomes. The lines *a–h* indicate the regions of the transverse sections shown in Figs. 11–15 and 21–23 of Plate 2.

Figure 5. Left side of one of the youngest larvæ. A preserved specimen 6 mm. long with 22 gill-slits and 51 . 13 = 64 myotomes. The caecum is not yet formed.

Figure 6. Ventral view of anterior region: from an immature preserved specimen having the pre-oral hood expanded.

Figure 7. Transverse section of brain cut 12 μ posterior to its anterior tip; showing the pigment bordering this end of the ventricle.

Figure 8. A section of the same series 40 μ posterior to the tip of the brain, where the ventricle is at its widest.

Figure 9. From the same series, 52 μ posterior to tip, where the ventricle is coming rapidly to a close.

Figure 10. Left side of the anterior extremity of a mature specimen macerated in Haller's liquid. The relative positions of lymph spaces and canals are shown. The dotted line is the anterior limit of the muscle fibers of the first myotome.

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PLATE XIV.

Figure 11. Transverse section of anterior end of body near extreme tip of notochord, at line (*a*) Fig. 4.

Figure 12. Transverse section at anterior end of pre-oral chamber, line (*b*) Fig. 4: the pre-oral pit, ciliated ridge and blood vessel are shown on the animal's right, the tips of some cirri within the chamber.

Figure 13. Transverse section at the posterior part of the pre-oral chamber, line (*c*) Fig. 4, showing the right ciliated ridge and blood vessel of the *Räderorgan*.

Figure 14. Transverse section of the middle of the body, at (*d*) Fig. 4, showing the position of the ovary on the animal's right side. The pharyngeal basket and the lining of the atrium are indicated diagrammatically.

Figure 15. Transverse section at (*e*) Fig. 4, where the atrium is ending in a spout, the free tip of which is shown. Two post-atrioporal pouches are seen within the coelome.

Figure 16. Grouping of muscle fibers in the right and the left sub-atrial ridges of a young larva; from longitudinal, horizontal sections. The limits of a myotome are marked by crosses.

Figure 17. Optical longitudinal section of three dorsal fin-rays and fin-ray spaces, from a preserved specimen stained alive by Bismarck brown. Certain large cells at the top of each chamber and parts of two dorsal lymph canals are shown.

Figure 18. Right ciliated ridge, pre-oral pit and anterior end of pre-oral chamber seen in horizontal, longitudinal section.

Figure 19. Optical, longitudinal section of the free part of a pre-oral cirrus with its axial skeletal rod. From a preserved specimen.

Figure 20. Transverse section of the anterior union of the ventral, metapleura-like ridges bounding the edges of the pre-oral chamber.

Figure 21. Transverse just anterior to anus, at line (*f*) Fig. 4, showing the median fin extending down from the right towards a median position.

Figure 22. Transverse section at the anus, (*g*) Fig. 4, with the median fin still on the right of the animal.

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Fig. 1

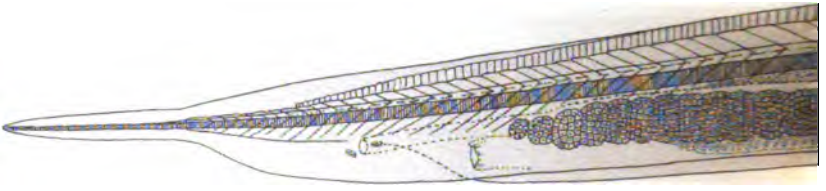


Fig. 2

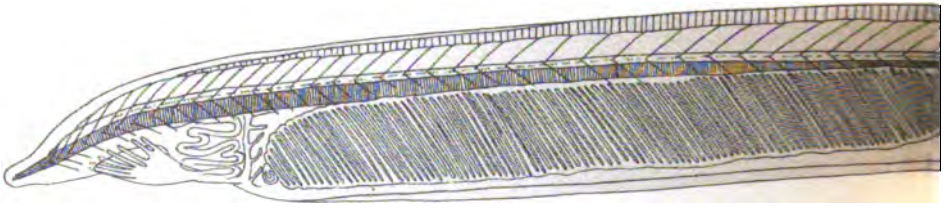


Fig. 3

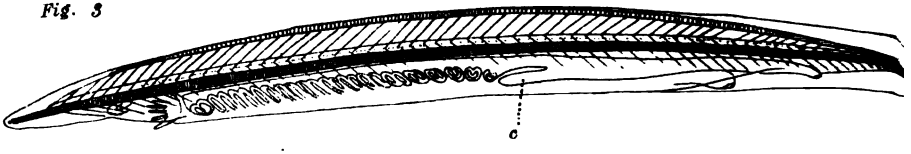


Fig. 4

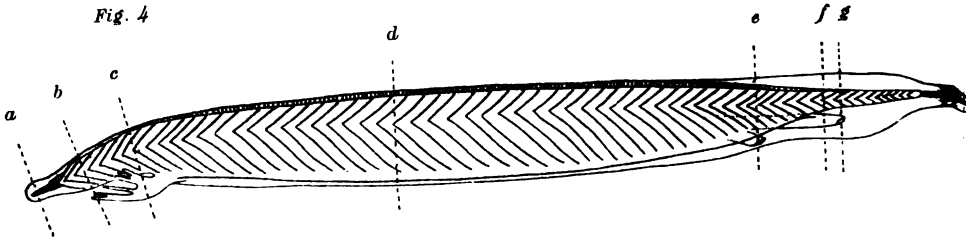
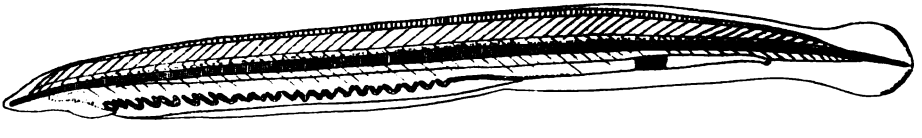


Fig. 5



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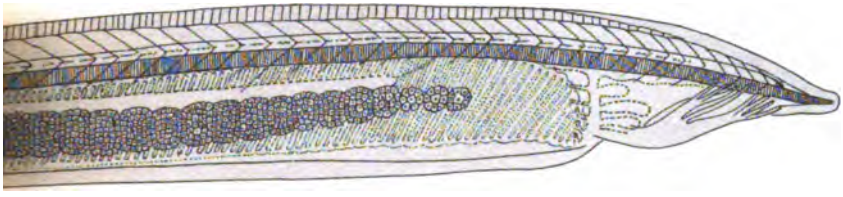

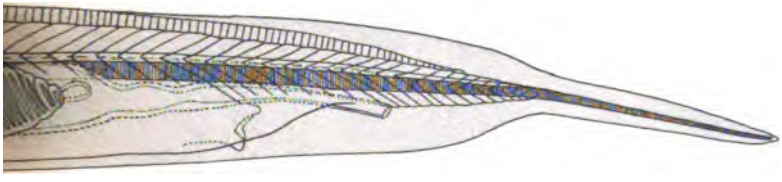

Fig 6

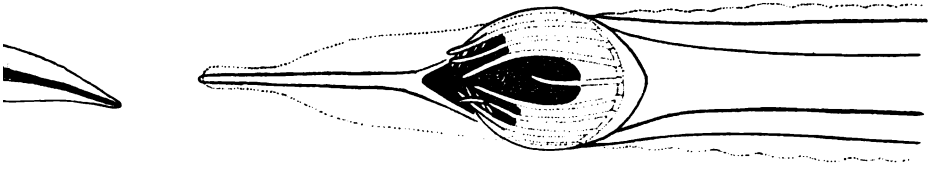

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Fig. 7

Fig. 8

Fig. 9

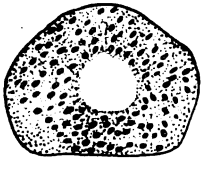

Fig. 10

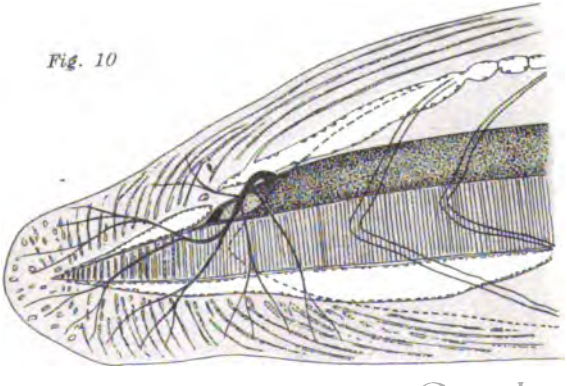

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Fig. 11

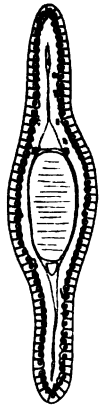

Fig. 12

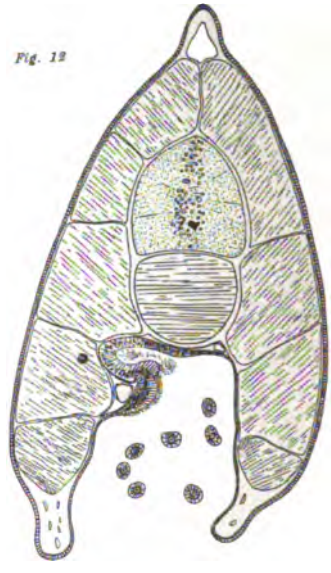

Fig. 13

Fig. 16

Fig. 17

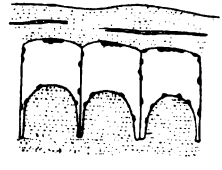

Fig. 18

Fig. 21

Fig. 22

Fig. 23

Fig. 1

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Fig. 14

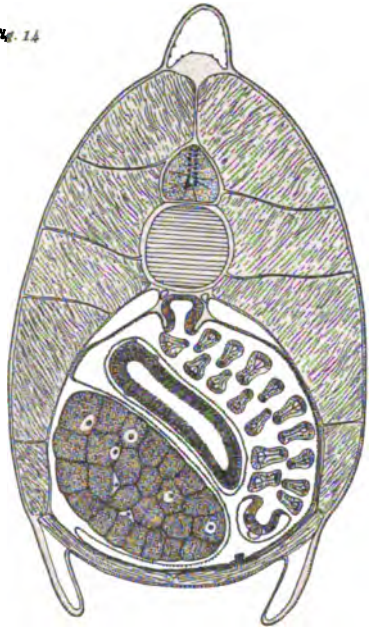


Fig. 16

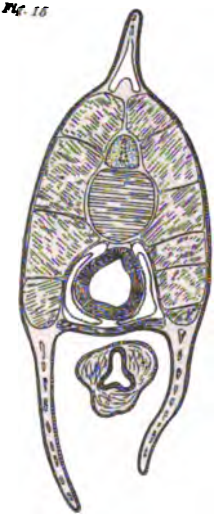


Fig. 19

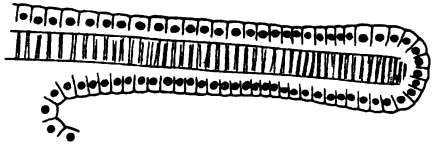


Fig. 20

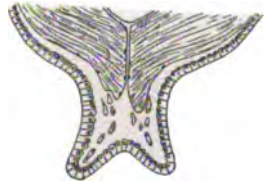
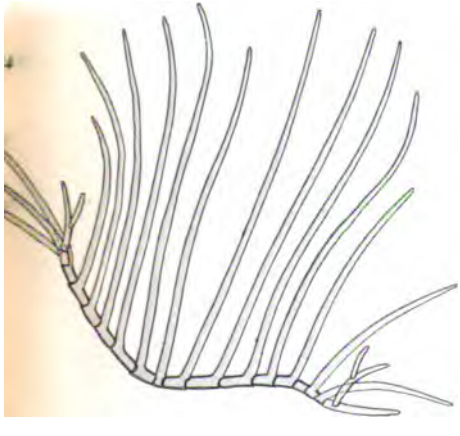


Fig. 25



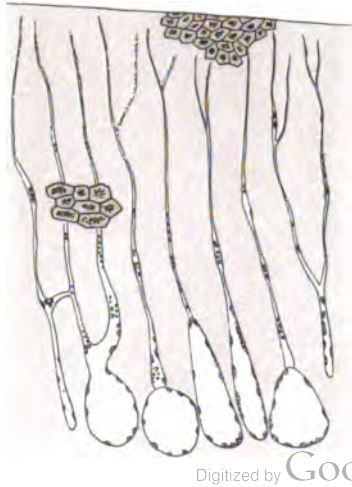


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Figure 23. Transverse section of the caudal process near its tip, (*h*) Fig. 4, showing existence of nerve cord and notochord, but the absence of muscles.

Figure 24. Skeleton of the pre-oral hood, somewhat flattened out. From macerations in Haller's liquid.

Figure 25. Optical, longitudinal section of the dorsal fin about the region (*g*) Fig. 4. Incipient fin-ray spaces and radiating lymph canals as seen in a preserved specimen stained by Bismarck brown when alive. Some epidermal cells containing colored spherules are shown at a higher focus.

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CONTRIBUTIONS TO THE EMBRYOLOGY OF CHITON.

By MAYNARD M. METCALF. With Plates XV and XVI.

I.

The material for these studies was collected and the early stages observed during the Summer session of the Chesapeake Zoölogical Laboratory at Jamaica, W. I., in 1891. The further study has been carried on in the laboratories of the Johns Hopkins University and of the United States Fish Commission at Woods Holl, Mass. I would express my gratitude to Colonel Marshall McDonald, Commissioner of Fish and Fisheries, and to Mr. Richard Rathbun, the head of the scientific division of the commission, for admission to the finely-equipped laboratory at Woods Holl and for many courtesies. To Professor T. H. Morgan, I am indebted for his invaluable kindness in preserving embryos for me during several days at Jamaica when I was sick and unable to attend to them myself. To Professor Brooks, my instructor, during the last four years, I am indebted, not so much for immediate assistance in the work here recorded as for the inspiration which led me to the work, and for constant suggestions giving broader views of kindred problems. I cannot too warmly express my gratitude and appreciation of his many kindnesses.

Along the rocky shores of the Island of Jamaica and upon the coral reefs several species of Chiton are abundant. Chiton marmoratus, Gmel., and Chiton squamosus, L., the species studied, are exclusively littoral. Chiton squamosus, the most common, abounds under the stones near low water line; as many as twenty individuals having been found under one stone. A smaller number are found crawling upon the surface of the wave-washed coral rocks of the shore, between tides. In this latter situation alone was Chiton marmoratus to be found. This species is not so plentiful, a search of an hour's time bringing to light not more than thirty individuals.

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Of the many hundreds of individuals of nine different species[1] observed during June and the early part of July, none were found bearing bunches of eggs in the mantle chamber. This habit observed by Kowalevsky in "Chiton Polii"[2] is therefore not characteristic of all species, at least in tropical waters. Males of most species, containing mature spermatozoa, were abundant. On the contrary, but two females, one Chiton marmoratus and one Chiton squamosus, laid eggs in the aquaria, though many were collected at different times and kept for several days.

Just before the ovulation commenced, the female began to crawl rapidly about the floor and sides of the aquarium. The male showed evidence of sexual excitement, being even more active than the female. Before the deposition by the latter of the first eggs the semen was observed to pour as a white, cloudy stream from beneath the posterior part of the mantle of the male, in the region of the orifice of the vas deferens. About a minute or a minute and a half later, the minute pea-green eggs commenced to be ejected from the corresponding point of the mantle chamber of the female. During the ordinary respiration of the Chitons, at one anterior point and at a posterior point on the opposite side, a small tube is formed by the arching up of the mantle edge, the bottom of the tube being formed by whatever surface the mollusc is resting upon. A constant stream of water passes into the anterior tube, through the mantle chamber and out of the posterior tube. During the discharge of the sexual products, instead of one there are two posterior tubes, one on each side, in the region of the orifice of the oviduct, or vas deferens, as the case may be. The eggs, or

[1] These species, which Dr. W. H. Dall very kindly identified for me, are:
Chiton marmoratus, Gmel.
Chiton squamosus, L.
Acanthopleura picea, Reeve. (Chiton piceus).
Acanthochiton spiculosus, Reeve.
Acanthochiton fascicularis, Monter.
Ischnochiton limaciformis, Reeve.
Ischnochiton funiculatus, Carp.
Ischnochiton sanguineus, Reeve.
Ischnochiton sp.

[2] A. Kowalevsky, "Embryogenie du Chiton Polii (Phillippi) avec quelques remarques sur le developpement des autres Chitones," in Ann. du Mus. d'Hist. Nat. de Marseille, Zool., Tome I, Memoire No. 5.

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spermatozoa, are carried out of the mantle chamber through these tubes by the ordinary respiratory current. At other times during ovulation the whole posterior part of the mantle of the female would be raised from the floor of the aquarium and the eggs allowed to pass out freely through the wide space thus formed. The eggs are fertilized after extrusion. Neither during the period of sexual excitement preceding the ejection of the sexual products by the two individuals, nor during the time the eggs were being fertilized, did the male and female come into contact with each other, or approach nearer than usual. Whatever exciting influence passed from one to the other was conveyed a considerable distance through the sea water of the aquarium. The extrusion of eggs by the female and of spermatozoa by the male, in each case observed, continued for two hours or more. This fact, probably, accounts in part for the different stages of development noted in any pipetteful of eggs collected.

The eggs are small, one-fifth of a millimeter in diameter, exclusive of the chitinous envelope. They are very opaque; those of Chiton squamosus more so than those of Chiton marmoratus. Each have a light pea-green color, which is of great assistance in manipulating them, rendering them easily visible and distinguishing them from particles of dirt. The chitinous envelope has a pattern peculiar to each species.

The spermatozoa have the form shown in Figure XXIV, Plate XVI. Usually the tail is attached to the blunt end of the head. Many cases, however, were observed, both at Jamaica and at Woods Holl, where the tail was attached to the pointed end of the head. Similar relations are by no means unknown in the spermatozoa of Mollusca. (Compare the condition in Mytilis [Kellogg[1]].) I have been unable to observe the process of fertilization and so can not say whether the two forms of spermatozoa are equally effective.

Methods. The embryos were studied alive but owing to their opacity and the presence of the chorion with its branched processes little could thus be learned in regard to the early stages except the time of the occurrence of some of the principal phenomena, e. g.

[1] J. L. Kellogg. "A Contribution to our Knowledge of the Lamellibranchiate Mollusks." Bulletin of the U. S. Fish Commission, 1890, Plate LXXXVIII, Fig. 67.

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gastrulation, formation of the velum. The external characters of the free-swimming larvæ and of the young chitons attached to the bottom can best be studied in live specimens. Many individuals in all stages of development were fixed in Kleinenberg's Picro-Sulphuric acid, stronger solution; in Pirenji's Chromo-Nitric acid; in an aqueous solution of Picric acid with sufficient Sodium Chloride added to bring the solution to the density of sea water, and in Corrosive Sublimate (aqueous solution). Of these the Picric-Salt solution gave, perhaps, the best results. After the usual hardening with alcohol, the latest stages could be stained and sectioned without further treatment; Borax-carmine, or Haematoxylin giving good results. The earlier stages require special treatment, first to remove the chorion, and second to remove the yolk with which all the cells are crowded. Eau de Labarraque (Hypochlorite of Soda) is effective for both, though I did not use it in the ordinary way. The embryos were passed from water into the ordinary, commercial Eau de Labarraque, cold and full strength; after one-third to three-quarters of a minute they were removed to water where the chorion soon swelled to more than twice its usual size owing to the dialytic action of the solution within the chorion with the circumambient water. The chorion could then be removed by agitation of the water, or by currents from a pipette.

The Sodium Hypochlorite, if allowed to act long (three minutes or more) on the embryos completely dissolves them. It acts, however, much more rapidly upon the yolk than upon the protoplasm and nuclei. By regulating the time of immersion one can obtain embryos with the yolk almost wholly dissolved and the protoplasm and nuclei nearly uninjured. These may then be stained and sectioned with ease, giving satisfactory results. The method is a crude one, but is the only way in which I have been able to successfully get rid of the yolk. Longer immersion in a weaker solution is not so successful, the protoplasm being much more injured.

In staining early stages, after treatment with Eau de Labarraque, the embryos were placed in weakly acidulated water and passed from this into Delafield's Haematoxylin. The acid washes out from the protoplasm before it does from the yolk granules, so, by regulating the time of immersion, one may obtain preparations with well-stained nuclei and cell boundaries while the yolk granules are unstained.

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Wax reconstructions from serial sections were made of embryos in the 24, 32, 36 and 40 cell stages and of different stages of gastrulation.

The opacity of the eggs and the presence of the chorion with its branching processes render any satisfactory study of the phenomena of maturation and fertilization impossible. Previous to division, the polar bodies are formed. These are usually two (Fig. II): sometimes one of these again divides into a larger and a smaller moiety; there being in these cases three pole bodies, one of normal size, one a third smaller, another two-thirds smaller than normal. I have twice in sections observed a nuclear spindle in the first polar body while the egg showed the spindle for the formation of the second polar body. In a few instances, even after the first division has taken place, only a single polar body of normal size can be observed. It is, of course, possible that such eggs are unfertilized.

CLEAVAGE.

External phenomena.

After segmentation begins it proceeds very rapidly with intervals of about ten minutes between the divisions. The first phenomenon observable is a slight flattening of the egg beneath the polar bodies, therefore at the animal pole (Fig. I, Plate XV). This flattening becomes more marked and in a few moments a slight furrow appears across the flattened area (Fig. II, Plate XV). This furrow deepens and at the same time rapidly extends around the egg, its two ends meeting at the vegetative pole (Fig. III, Plate XV). By further deepening of the furrow the egg is divided into two blastomeres of equal size (Fig. IV, Plate XV). Each blastomere is as nearly spherical as the limited space within the chorion will allow. *Two cells.*

Previous to the next division, about seven minutes after the separation of the first two blastomeres, the blastomeres become pressed together till the two together form a nearly spherical embryo (Fig. V, Plate XV). A similar phenomenon is observed previous to each of the first few divisions. In later stages, when the cells become smaller, it can not be perceived. This appression of the blastomeres occurs near the end of the "period of rest," there-

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fore preceding the next, rather than following the former, division. A second meridional furrow now appears, dividing the embryo into four blastomeres of similar size and shape (Fig. VI, Plate XV). I am unable to say whether the second furrow commences at the animal pole or not, but from analogy with the first furrow and from the position of the nucleus of each of the two first blastomeres (as will be seen later) it is probable that it does so. *Four cells.*

The third furrow is parallel to the equator and divides the embryo into four large vegetative cells (V) and four slightly smaller animal cells (A) (Fig. VII and Fig. VIII, Plate XV). The two cells formed from one blastomere of the four cell stage at first lie in the same meridian. (The nuclear spindle is vertical, so far as I can determine from sections.) An immediate shifting occurs by which the animal cells are brought into meridia intermediate between those of the vegetative cells. The direction of this shifting I have been unable to determine by direct observation. *Eight cells.*

In the two species studied no twelve-celled stage occurred, though in Chiton Polii Kowalevsky found a twelve-celled embryo.

By a further division, neither meridional, nor equatorial, but oblique, the eight-celled embryo is divided into sixteen blastomeres (Fig. IX and Fig. X, Plate XV). The eight new cells are much smaller than their parents, being budded off, as it were, from them toward the equator. The small cells are all of the same size and are elliptical in surface view. Viewed from the upper pole, the animal cells bud off their small cells (a^1) in a left handed spiral; i. e. in a direction opposite to that taken by the hands of a watch. The vegetative cells also, when viewed from the animal pole, bud off their small cells (v^1) in a left handed spiral. [It will be convenient to speak of all subsequent spiral divisions or shiftings of the blastomeres as if viewed from the animal pole, thus referring them in a constant manner to the main axes of the embryo. In reality the embryo is so perfectly opaque that the cells of the vegetative portion can not be seen at all from the upper pole.] *Sixteen cells.*

The fifth division affects the vegetative half of the embryo before it is visible in the animal portion. It therefore may be divided into two parts, $5v$ the division in the vegetative half of the embryo, and $5a$ the division in the animal half of the embryo.

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$5v$ (Fig. XI and Fig. XII, Plate XV). The large vegetative cells (V) each bud off a small cell (v^2) toward the vegetative pole. These are pear-shaped in surface view, their larger ends being at the centre of the vegetative pole of the embryo, their emarginate ends lying somewhat between the large vegetative cells. The division $5v$ is followed by a shifting of the cells v^2 in the direction of a left handed spiral [as viewed from the animal pole]. $5a$ (Fig. XI and Fig. XIII, Plate XV). This division commences a little later than the former division, but before it is complete. The large animal cells (A) each bud off a small cell (a^2) similar in shape to the cells v^2, of nearly the same size, and having a similar position with reference to the animal pole. This division is followed by a shifting in the direction of a left handed spiral. *Twenty-four cells.*

There follows next a division in both the animal and vegetative halves of the embryo which I have been unable to observe. I can but describe the form and position of the eight new cells and cannot with absolute certainty determine their parentage. Up to this time, we have seen, in the equator of the embryo, eight cells arranged in pairs (Fig. IX and Fig. XI, Plate XV), each pair containing one cell from the animal half of the embryo (a^1) and another from the vegetative half of the embryo (v^1). After the division, which I have not observed, there are found four cells in place of each former pair (Fig. XIV, Fig. XV and Fig. XVI, Plate XV). (The figure shows the remarkably regular condition in many embryos. In other embryos the cells are more or less irregular in shape.) The view that these four cells result from a division of the two cells formerly having the same position is supported by several facts: (1) The position of the four is the same as that of the former pair: (2) Each of the four cells is about half the size of one of the former two (if we take into account differences in shape and in the amount of surface exposure in the four cells, they are probably all equal in size): (3) The cells a^1 and v^1 have not previously divided: (4) There are no other cells from which the new products could arise without causing a break in the general symmetry of the division. If (Fig. XIV, Plate XV) the cell v^4 arose from V the division was meridional. If the cell a^4 arose from either of the cells A it must have been by a division approximately parallel to the equator. The cell a^4 could not arise from

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the cell a^2, for it is larger than the cell a^2 and the cell a^2 is no smaller now than when first formed. There seems then no room for reasonable doubt that the eight new cells produced by this division arose from the eight equatorial cells a^1 and v^1. Accepting then this interpretation, we may also say that the cell a^1 (Fig. IX) has given rise to the two cells a^3 and a^4 (Fig. XIV), both of them lying in the animal half of the embryo, and that the cell v^1 (Fig. IX) has given rise to the two cells v^3 and v^4 (Fig. XIV), both of them lying in the vegetative half of the embryo. *Thirty-two cells.*

In the thirty-two-celled stage, then, we find present two sets of four large cells each (A and V) and six sets of four small cells each, one set of four at each pole and four sets equatorial. The equatorial quartettes lie each above one of the large vegetative cells and between two large animal cells (Fig. XIV).

The next division affects only the vegetative half of the embryo. The small cells v^3 each divide into two cells of unequal size and different shape (Fig. XVII and Fig. XVIII, Plate XV). The division occurs in a right-handed spiral, as is indicated by the nuclear spindle (Fig. XVII). Of the two cells which result from the division of one of the cells v^3 one, the larger, v^5, is pear-shaped like its parent and retains the former position; the other is spheroidal and lies nearer the centre of the vegetative pole in a meridian forty-five degrees to the left (as seen from the animal pole) of that occupied by v^5. We will call this cell v^6. The eight small cells, v^5 and v^6, lie in a hollow between the four cells V. This hollow is the bottom of the future gastrula cavity. *Thirty-six cells.*

The thirty-six-cell stage exactly resembles that figured by Kowalevsky for "Chiton Polii," but the pattern which Kowalevsky represents for the animal pole is, in Chiton marmoratus and Chiton squamosus, the pattern of the vegetative pole, while his figure of the vegetative pole of "Chiton Polii" corresponds to the pattern of the animal pole of Chiton marmoratus and Chiton squamosus. This is shown very plainly, both in surface view and in wax reconstructions made from serial sections. The next division, the last previous to gastrulation, affects only the four large cells A in the animal half of the embryo. Each of these buds off toward the animal pole a cell (a^5, Figs. XIX–XXI, Plate XVI) intermediate in size between itself and the small cells a^3. This division

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is exactly meridional and the resulting cells retain their original position, *i. e.*, no subsequent shifting occurs. *Forty cells.*

At this stage the cells *V* are still very large compared with the neighboring cells. The cells *A*, though still large, are much smaller compared with the cells which have arisen from them. This difference is due to the fact that the divisions in the vegetative half of the embryo have been more unequal than in the animal half, the cells *V* having budded off relatively smaller portions of themselves than did the cells *A*. This may be attributed to the presence of a greater amount of food yolk in the large cells *V*.

We have heretofore referred the segmentation to the embryo as a whole. A few words of comparison between the phenomena in the animal and in the vegetative portions of the embryo will be profitable. A comparison of the figures shows that, with the exception of the manner of formation and the position of the cells a^5 and v^6, one half of the embryo is almost the exact counterpart of the other. In the vegetative half of the embryo the cells v^6 are formed from the cells v^3 and lie at the vegetative pole. They are formed by a spiral division, therefore they lie in meridia intermediate between the cells v^5; *i. e.* in the meridia in which the cells v^3 originally lay before they shifted their position. In the animal half of the embryo the cells a^5 are formed directly from the cells *A* in the meridia in which the cells a^2 had been formed and they retain this position. They have the same position then in reference to the animal pole that the cells v^6 have in reference to the vegetative pole, save that the latter lie directly at the vegetative pole, while at the animal pole are found the cells a^3 in an inner zone, the cells a^5 being outside them. This difference serves as a criterion for orienting the egg, since at the vegetative pole the cells of the inner circle are in the same meridia as the large cells *V*, while at the animal pole the cells of the inner circle are in meridia intermediate between the meridia in which lie the large cells *A*.

With the exception then of this point the divisions and subsequent shiftings in the animal and vegetative halves of the embryo correspond; the shifting in the one case, however, being exactly in the opposite direction to that in the other case. Thus, when we compare the view of the animal pole with the view of the vegetative pole, the cells a^1 have been shifted forty-five degrees to the left of

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their original position, while the cells v^1 have shifted forty-five degrees to the right and so on for each succeeding division. This is readily seen to be consequent upon two facts. 1. In the first shifting which took place in the eight cell stage, the cells of the two halves of the embryo twisted in opposite directions. 2. In the subsequent divisions and shiftings none of the products of one of the cells A pass over into the quadrant of another of the cells A, and a similar rule applies to the vegetative half of the embryo. Thus the cells v^3 arise from the cells V and shift forty-five degrees to the left. The cells v^6 arise from the cells v^2 and shift forty-five degrees to the right, returning to the meridia from which their parents, the cells v^2, were removed. Each cell, then, lies in the same meridian as its grandparent—a fact shown more clearly in the cleavage of such eggs as those of Nereis[1] and Crepidula.[2]

We notice that most of the divisions are of the radial type. In certain cases, however, the cells, instead of dividing meridionally and subsequently shifting, divide in the first case obliquely reaching their definitive position without any subsequent shifting, e. g. the division of the cells v^2 (Fig. XVII, Plate XV). It seems that this is a coenogenetic modification of the radial type of segmentation. It may be due either to the simplification of development by acceleration, or to crowding because of the presence of the larger yolk-filled cells which would interfere with the necessary freedom of motion in the many shiftings.

Kowalevsky describes a twenty-two cell stage as occurring in "Chiton Polii." Such a stage, if found, would be an anomalous break in the regular sequence of segmentation as found in Chiton marmoratus and Chiton squamosus. It seems possible that Kowalevsky's observation was made upon an abnormal embryo.

Internal Phenomena.

In the unsegmented egg the nucleus is surrounded by a mass of protoplasm quite clear from yolk. The narrow outer zone of the egg is also composed of comparatively yolk-free protoplasm. All

[1] E. B. Wilson. The "Cell-Lineage of Nereis" in the *Journal of Morphology*, Vol. VI, No. 3, July, 1892.

[2] E. G. Conkling. "The Cleavage of the Ovum in Crepidula fornicata," in Zool. Anz., XV, No. 391, May, 1892.

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the rest of the egg is so filled with yolk granules that not even protoplasmic threads can be discerned. There is a delicate closely fitting membrane around the egg which stains with haematoxylin. In later stages it is seen to clothe the blastomeres, forming the cell walls.

Previous to the first division the nucleus assumes an excentric position lying just below the animal pole. This is in all probability associated with the flattening of the animal pole of the egg and the fact that the first furrow begins at the animal pole. The nucleus remains in the animal end of the first two blastomeres during their division into four; therefore it is probable that the second furrow, like the first, commences at the animal pole of the egg. In each blastomere of the four-celled stage the nucleus lies nearer the animal than vegetative pole, a fact which probably is correlated with the unequal division of each blastomere into one larger and one slightly smaller cell.

It is readily seen that in the unsegmented egg, just previous to its first division, the nucleus is much nearer to the animal pole than it is in one of the blastomeres of the four-celled stage. It would be interesting to determine why this is so. If we seek a mechanical explanation (a phylogenetic significance in the phenomena of segmentation is shown to be improbable by a comparison of the segmentation of the eggs of Crepidula and of Nereis and especially by E. B. Wilson's work upon the segmentation of Amphioxus[1]) I could only suggest that the nucleus of each of the four blastomeres is more than one-fourth as large as the nucleus of the unsegmented egg and that it is therefore better able to manipulate the yolk. It is well known that the cell protoplasm in the later periods of development in many animals increases at the expense of the yolk. It is very improbable that within the twenty minutes required for the first two divisions the protoplasm of the Chiton egg has increased to any considerable extent at the expense of the yolk. We can not suppose that in the four blastomeres the protoplasm is a so much larger proportion of the whole, that the nucleus is on that account able to assume a more central position. It seems reasonable, then, to suppose that the nuclei themselves,

[1] E. B. Wilson, "On Multiple and Partial Development in Amphioxus;" in Anatomischer Anzieger, Oct. 1892.

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or, more properly, the essential elements of the nuclei have increased in bulk during the segmentation. It would be profitable to make most careful observations upon this point in eggs less difficult to study.

In the four-celled stage the segmentation cavity is already present.

The internal phenomena of cleavage, as well as the study of external appearances, show that in the two species studied no twelve-cell stage occurs. For example, in one eight-celled embryo of Chiton marmoratus the nuclei of the four larger cells and of one of the animal pole cells were in the same stage, an early stage, of division, the chromatin threads not yet separated from the equator of the spindle. The nuclei of the three other animal pole cells showed no traces of division. The segmentation cavity of an eight-celled embryo, as seen in oblique section, is shown in Fig. XXX, Plate XVI.

The eight equatorial cells of the sixteen-cell stage do not extend in to the segmentation cavity, but are more superficial, lying in the grooves between the outer surfaces of the large cells *A* and *V*.

There is little else of interest to be seen in sections of embryos previous to gastrulation.

Gastrulation.

In the forty-cell stage gastrulation has already commenced. We have seen that the cells v^5 and v^6 lie at the bottom of a hollow between the four large cells *V* (Fig. XVIII, Plate XV). This hollow is part of the future gastral cavity. According to Kowalevsky's description for "Chiton Polii" the region of the embryo which invaginates to form the gastrula cavity has its cells arranged in a pattern exactly resembling the pattern of the cells of the animal pole of Chiton squamosus and Chiton marmoratus; while the pattern he describes for the animal pole of "Chiton Polii" corresponds to the pattern of the cells in the vegetative half of the embryo of either of the species I have studied. It seems strange that there should be such a difference between species, yet I hesitate to suggest that Kowalevsky's observation was mistaken, for his memoir is beautifully accurate, as my study of closely related species has shown me.

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Division continues as gastrulation proceeds. First the large cells *V* each separate into two nearly, if not exactly, equal cells *V* and v^7 by a division parallel to the equator (Fig. XXII and Fig. XXIII, Plate XVI). By this division of the four cells *V*, the four of the eight resulting cells which lie nearer the vegetative pole (v^7) are pushed further toward that pole; the gastral invagination, from the same cause, becoming deeper. At about the same time as this division in the vegetative half of the egg the four small animal pole cells a^5 divide giving rise to eight cells a^6 and a^7 (Fig. XXIV, Plate XVI). The complete opacity of the embryo and the small size of the individual cells prevent any further accurate study of the cell-lineage within the animal half of the embryo. With objectives of sufficiently high power for the study of the minute blastomeres of such small embryos it is impossible to get sufficient surface illumination.

In the vegetative half of the embryo the cells are larger than in the animal half. While division is proceeding in the animal half of the embryo we find each of the cells *V* dividing by a meridional furrow into two equal cells v^8 and v^9 (Fig. XXVI, Plate XVI), the four cells v^7 being pushed into the gastral invagination. We see four small cells (marked ?) lying between and a little below the four pairs of cells $v^8 + v^9$. These are apparently the four cells v^5 which formerly lay between the polar ends of the large cells *V*, but which during the early stages of invagination have pushed back a little and now lie between the adequatorial ends of the cells v^7.

In an embryo somewhat older these cells are still shown (Fig. XXVII, Plate XVI), one of them having divided into two. This embryo is very interesting. We see the blastopore is now considerably smaller than before and there is a remarkable club-shaped furrow along what we shall see later becomes the mid-ventral line. This furrow is shallower behind and deeper at its anterior enlarged end. Counting the blastopore as the posterior part of the ventral furrow the latter may be said to be dumb-bell shaped with an anterior and a posterior enlargement. At the point where the furrow leads into the archenteron lie the two cells (products of the division of one of the cells marked "?" in Fig. XXVI) at the bottom of the furrow. The four large endodermal cells, v^7, can still be made out deep in the gastral cavity, though they are now

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overgrown by the eight cells v^8 and v^9 (Compare Fig. XXVI). This embryo is bilaterally symmetrical, the antero-posterior plane being indicated by the mid-ventral furrow and the division of one of the small cells ("?") into two cells.

The blastopore narrows more and more, the cells v^8 and v^9 growing over the five small cells. At the same time the four large endodermal cells, v^7, each divide by a meridional furrow into two cells; all the products being equal so far as can be seen from surface view (Fig. XXVIII, Plate XVI). The ventral furrow still persists, but is less marked, and its anterior enlargement can not be seen. As the blastopore narrows, the ventral furrow disappears. As the blastopore moves forward to become the mouth we shall find the furrow reappearing. Fig. XXIX, Plate XVI, shows an embryo in which the blastopore has reached its smallest dimensions previous to migration. The endodermal cells are now completely enclosed and no longer appear in surface view. The blastopore has a peculiar shape. It is a short antero-posterior slit which enlarges behind to a circular hole from which push out two lateral arms.

The later history of the blastopore is in many respects remarkable (Fig. XXX, Plate XVI, 3–7). Its posterior enlargement disappears (4), the connection of the archenteron with the exterior being limited to the anterior end of the former slit-like blastopore. The blastopore moves still further forward, coming to lie just behind the velum (5–7), in the region formerly occupied by the anterior pit of the dumb-bell-shaped ventral furrow. Traces of the ventral furrow can be seen behind the blastopore, as a shallow groove along the path of its migration. This soon entirely disappears. Its presence for a time, and especially the persistence for a short period of the posterior pit of the ventral furrow (4) after the blastopore has moved forward, shows that we have to do with an actual migration of the blastopore and not with an excessive growth of the dorsal part of the posterior region of the embryo pushing forward the blastopore. The somewhat similar migration of the blastopore in Patella seems, according to Patten,[1] to be due to the latter cause.

[1] Wm. Patten, "The Embryology of Patella;" in "Arbeiten a. d. Zool. Inst. d. Univ. Wien, etc." Tome VI, 1886.

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To recapitulate the history of the blastopore (Compare Fig. XXX, Plate XVI). It forms at the vegetative pole. It is at first quadrangular with a club-shaped furrow extending from its anterior edge forward along the mid-ventral line to the velum.[1] This furrow disappears. The blastopore sends out an anterior slit-like process (i. e. the embryo appears to split along the mid-ventral line). The posterior portion of the blastopore closes, the anterior remnant moving still further forward to lie just behind the velum. A slight groove may be seen for a short time along the path through which the blastopore migrated.

The ectoderm around the blastopore pushes in to form the stomodaeum, the blastopore at the same time closing by the appression of its lips. (No perforation ever appears in sections at the apex of the stomodaeum in embryos of this age). The mouth forms by the direct re-opening of the blastopore.

The phenomena of gastrulation in Chiton at once suggest comparison with Peripatus, in which the blastopore is at first slit-like, then dumb-bell-shaped, the anterior and posterior enlargements persisting as mouth and anus. The question of interest is whether the resemblances between the processes of gastrulation in the two cases are fundamental or are secondary. I do not see that the question can be definitely answered. According to either interpretation there have been great secondary changes. I cannot see that the early appearing ventral furrow (a mere depression in the ectoderm) is any indication of a former dumb-bell-shaped blastopore occupying the same position. Of more significance is the fact that the blastopore is at one time slit-like with a posterior enlargement and that it closes from behind forwards, the anterior remnant later migrating further forward to the position of the definitive mouth. Whether these are secondary modifications or are related to the phenomena in Peripatus cannot be shown by study of the conditions in Chiton. It is certainly of interest that there are in Chiton these phenomena which may be interpreted upon the hypothesis of the primitive nature of the slit-like blastopore.

[1] At this time the velar cells are not ciliated but can be distinguished by their slightly greater size, but more by their regular arrangement in the characteristic double row.

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Sections of embryos in different stages of gastrulation show little of special interest except the manner of formation of the mesoderm which I find to be, in both species studied, exactly as described by Kowalevsky for "Chiton Polii." At the posterior edge of the blastopore the two outermost endoderm cells are seen to be greatly enlarged (Fig. XXXII and Fig. XXXIII, Plate XVI, *m*). They soon each divide into two cells, one of each pair remaining as part of the endodermal wall of the archenteron, the other lying in the now small remnant of the segmentation cavity. The later history of the mesoblast will be taken up in a future paper in connection with the development of the definitive body form and of the organs.

I would call attention to two groups of four cells each, seen in embryos in which the blastopore has not yet migrated from its original position (Fig. XXIX, Plate XVI, a postero-ventral view of such an embryo). They lie dorsal to the blastopore, on each side of the mid line. They are distinguished from the surrounding cells of the ectoderm by their greater size and by the fact that they protrude more from the surface of the embryo, their outer contour being more rounded than that of the neighboring cells. I am not yet prepared to make any suggestions as to the meaning of these two groups of cells, nor can I determine from what blastomeres they are derived.

EXPLANATION OF PLATES.

The different stages of segmentation and gastrulation in the two species studied are found by careful comparison to be identical, therefore each figure represents the condition in both Chiton marmoratus and Chiton squamosus. The figures are not all camera drawings nor are they mere diagrams, though individual variations in the shape of the blastomeres are not preserved. The figures show what may be called the typical condition as derived from a comparison of many individuals. The segmentation is so remarkably regular that many embryos show practically no deviation from the almost mathematically symmetrical condition indicated by the figures.

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Reference Letters.

A indicates one of the four animal pole cells of the eight-celled embryo, or the larger one of its descendants; a^1 to a^7 indicate the smaller descendants of the first large cells *A*. *V* indicates one of the four vegetative pole cells of the eight-celled embryo, or the larger one of its descendants; v^1 to v^9 indicate the smaller descendants of the first large cells *V*.

bl = blastopore.

m = large endoderm cell about to give rise to the mesoblast.

x = group of four peculiar cells dorso-lateral from the blastopore.

The reference letters are further explained in the text.

The magnification of all figures is about 300 diameters.

Plate XV.

Figs. I–XI are side views of segmenting embryos, the animal pole in each case being toward the top of the plate.

Figs. I–III, the first furrow appearing.

Figs. IV and V, two cells.

Fig. VI, four cells.

Fig. VII, eight-celled embryo seen from the meridian of the animal cell, *A*.

Fig. VIII, same embryo seen from the meridian of the vegetative cell, *V*.

Fig. IX, sixteen-celled embryo seen from the meridian of the vegetative cells, *V*.

Fig. X, same embryo seen from the meridian of the animal cell, *A*.

Fig. XI, twenty-four-celled embryo seen from the meridian of the animal cell, *A*.

Fig. XII, view of the vegetative pole of the same embryo.

Fig. XIII, view of the animal pole of the same embryo.

Fig. XIV, thirty-two-celled embryo seen from the side, in the meridian of the vegetative cell, *V*.

Fig. XV, view of the animal pole of the same embryo: the nuclei of the small cells to be produced by the next division but one are shown in the polar ends of the large cells, *A*.

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Fig. XVI, side view of the same embryo from the meridian of the animal cell, *A*.

Fig. XVII, view from the vegetative pole of an embryo about to enter on the thirty-six-celled stage by the division of the cells v^3, each of which now shows two nuclei.

Fig. XVIII, thirty-six-celled embryo seen from the vegetative pole. The division which was taking place in Fig. XVII is now complete.

Plate XVI.

Fig. XIX, embryo of forty cells, side view, from the meridian of the vegetative cell, *V*. (Compare Fig. XV, Plate I, in which the large cells, *A*, each show at their polar ends the nucleus of the cell, a^5, about to be formed.

Fig. XX, same embryo, side view, from the meridian of the animal cells, *A*.

Fig. XXI, view of the same embryo from the animal pole.

Fig. XXII, embryo of forty-four cells, side view, from the meridian of the animal cell, *A*.

Fig. XXIII, same embryo, side view, from the meridian of the vegetative cell, *V*.

Fig. XXIV, view from the animal pole, of an embryo of forty-eight cells, the cells a^3 having given rise to the cells $a^6 + a^7$.

Figs. XXV–XXVIII, gastrulating embryos seen from the vegetative pole.

Fig. XXV, view of the forty-eight-celled embryo, showing only the cells at the bottom of the archenteron and at the edge of the blastopore.

Fig. XXVI, an older embryo. The cells *V* (compare Fig. XXII) have divided into the cells $v^8 + v^9$. The cells v^7 are sinking into the archenteron.

Fig. XXVII, an older embryo. The ventral furrow is outlined by the dotted line.

Fig. XXVIII, an older embryo. The ventral furrow has disappeared.

Fig. XXIX, a postero-ventral view of an older embryo. The blastopore is now slit-like with a posterior enlargement. Two

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groups of large cells (x) are seen dorsal to the blastopore, on each side of the mid-line.

Fig. XXX, outlines of the blastopore and ventral furrow at different stages during gastrulation and the migration of the blastopore. The blastopore is denoted by the continuous line, the ventral furrow by the dotted line.

Fig. XXXI, section of an eight-celled embryo, showing the segmentation cavity.

Fig. XXXII, transverse section of an embryo at the close of gastrulation and before the migration of the blastopore. The section cuts the posterior edge of the blastopore. *m*, designates the large endoderm cells about to bud off the mesoblasts.

Fig. XXXIII, longitudinal vertical section of a similar embryo, showing one of the large cells, *m*, behind the blastopore.

Fig. XXXIV, spermatozoa. *A*, usual condition. *B*, condition seen in about a quarter of the spermatozoa.

One-celled stage, ...	Figs. I–III.
Two-celled stage, ...	Figs. IV–V.
Four-celled stage, ...	Fig. VI.
Eight-celled stage, ...	Figs. VII–VIII.
Sixteen-celled stage, ...	Figs. IX–X.
Twenty-four-celled stage, . .	Figs. XI–XIII.
Thirty-two-celled stage, . . .	Figs. XIV–XVII.
Thirty-six-celled stage, . . .	Fig. XVIII.
Forty-celled stage, ...	Figs. XIX–XXI.
Forty-four-celled stage, . . .	Figs. XXII–XXIII.
Forty-eight-celled stage, . . .	Fig. XXIV.
Gastrulation, ...	Figs. XXV–XXX.

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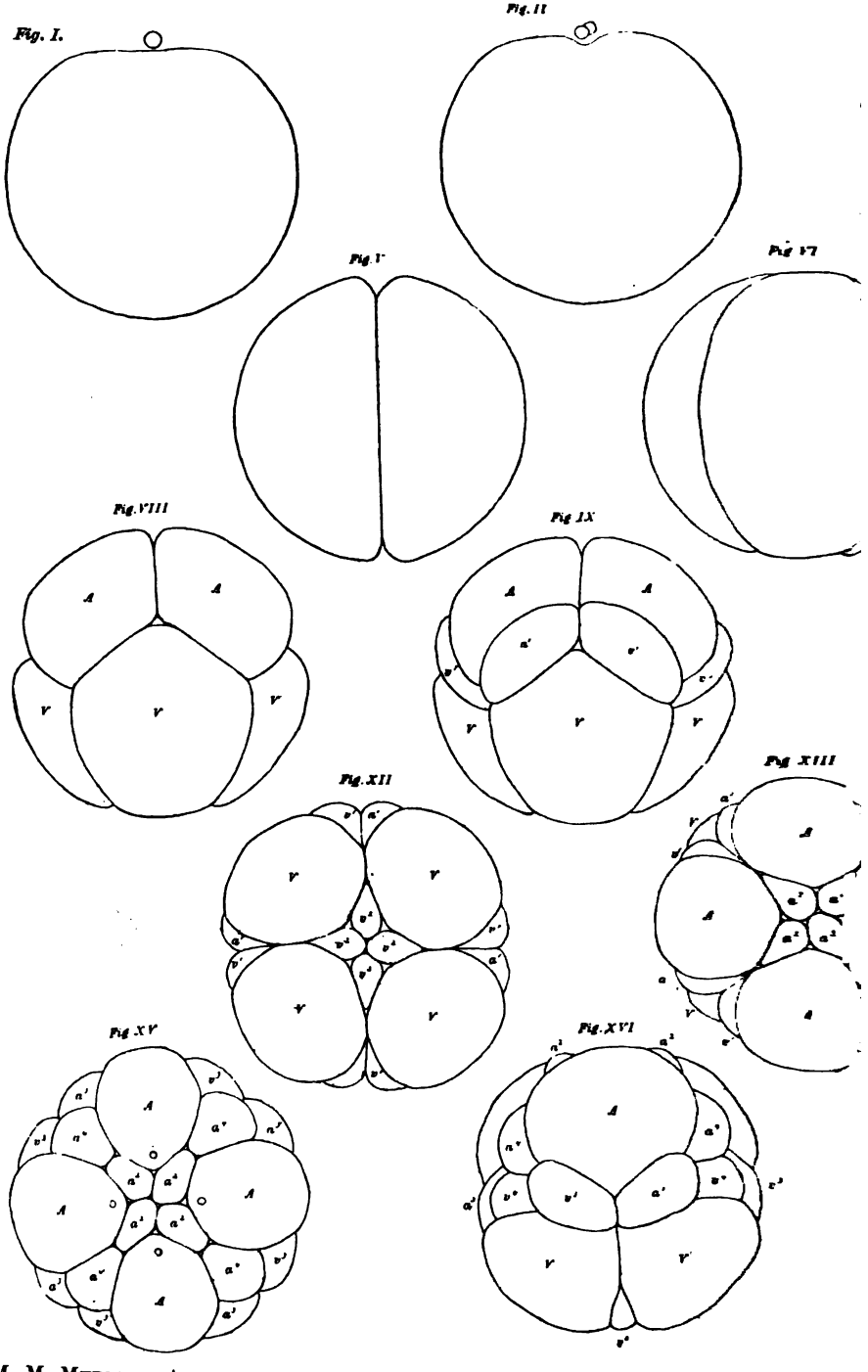

M. M. METCALF, AUTOGRAPH.

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Fig. XXI

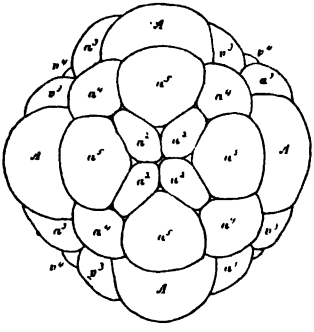
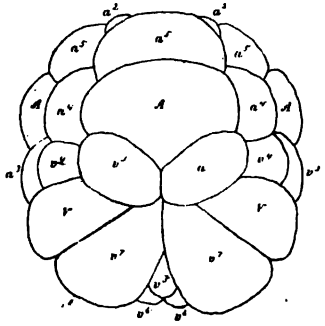


Fig. XXII



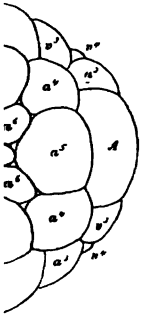


Fig. XXVI

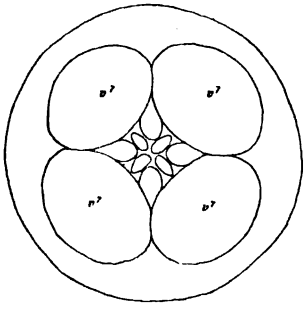


Fig. XXVIII

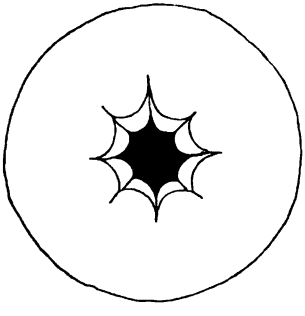


Fig. XXIX

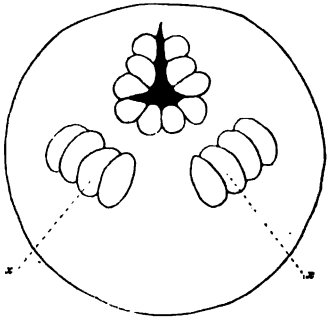


Fig. XXXIV

Fig. XXXII

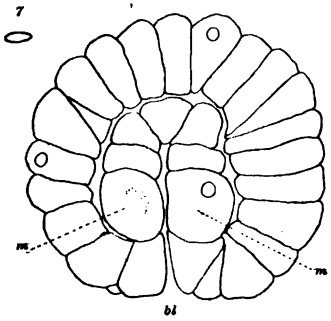
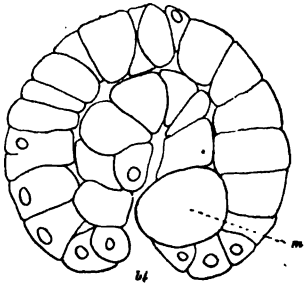


Fig. XXXIII



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THE FORMATION OF THE SO-CALLED CYPRESS-KNEES ON THE ROOTS OF THE TAXODIUM DISTICHUM, *RICHARD.*

By JOHN P. LOTSY, PH. D.

With Plates XVII and XVIII.

Every one who has visited one of the marshy places where the Taxodium distichum has its home, has been immediately struck by the difference in the appearance of this tree in such places as compared with the same tree when growing on high and dry soil. As we know the Taxodium distichum in botanical gardens, on lawns or in parks, growing in a soil elevated considerably above the water level the tree does not show any striking peculiarities, except its fine growth and beautiful foliage, on account of which it will always rank among the finest trees in existence. On these marshy grounds, however, its natural habitat, the tree shows the remarkable peculiarity of being surrounded over a large area by cone-shaped bodies rising perpendicularly from the soil, presenting about the same appearance as if a number of tentpins were driven in the soil only awaiting the ropes to which the tent has to be tied. These cones have been known for a long time and much has been written as to their probable function. European botanists (see Engler u. Prantl. Natürliche Pflanzenfamilien, II Theil, p. 28) say that it is not settled whether these organs are normal to the plant or whether they should be considered as pathological formations. Among American botanists, who, of course, have had a much better opportunity of studying these formations, the idea of their pathological nature has apparently been discarded and two different and diametrically opposite theories have been advanced which we might characterize as the mechanical and the physiological theory. The latter, which is apparently the most widely accepted one, is strongly defended by Wilson, who considers these knees as organs for the aeration of the sap in the submersed roots, and in favor of this theory is the fact that the trees do not form these knees on dry and elevated soil, but only on marshy ground. On the other hand I might remark, that I have seen many Taxodia in Europe from the Mediterranean shore upwards to the

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Holland coasts which were growing in land thoroughly soaked with water but which never formed any knees, yet the trees were beautiful old specimens of their kind.

The other theory suggested by Mr. Lamborn (see American Naturalist, 1890) does not consider the physiological theory as adequate, but calls attention to the fact that a tree of the size of the Taxodium elevated far above its mates, a giant of the forest, and exposed to the hurricanes of these regions, if placed in a soil composed of a yielding material would have a great advantage from the presence of these knees, since they would materially strengthen the anchor which the root makes at the point where this formation takes place, as we shall see later on. In view of these controversies between the advocates of theories so radically different, of which the one does not appear to be better founded than the other, it seemed to me that before undertaking to study the question of their function, a detailed research as to the formation and histological structure of these knees was indispensable and it was with pleasure that I made use of the opportunity to collect the necessary material for this purpose. It is the purpose then of this present paper merely to show the development of the Cypress-knees, and it has to be considered as the first part of an opus treating the matter in full. Regarding the function of these formations I am, as yet, entirely unprepared to give a definite opinion, and I will only state that the material I collected and preserved during the month of August, 1892, showed in its young stages in abundance and in its old ones in lesser number, the dark-brown spores of a fungus (Plate XVIII, Figs. 6, 7) with not a trace of mycelium except near the top of the knee where the mycelium was plainly visible (Fig. 8, Plate XVIII). As my material was preserved in strong alcohol and sublimate, attempts to germinate the spores failed, as was to be expected, so that the systematic position of the fungus is very doubtful, and I can only suggest that their occurrence inside of cells and the total disintegration of their mycelium seems to point towards a possible relationship with the group of the Ustilagineæ. It is far from my purpose to advance the idea that the growth of this fungus is the cause of the formation of the knees, the only way to decide this question is to obtain a pure culture and make infection experiments. An attempt to get a culture from fresh material obtained

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at the same spot this Spring failed on account of the very much greater scarcity of the spores at this season, so that the matter will have to wait until August, the time when they were collected last year. Similar spores have also been found in much older knees sent to me from Florida, by the courtesy of Mr. Lamborn, whom I thank very cordially for this. As the matter stands now, the fungus may just as well be considered an accidental occurrence in the knees as standing in any causative relationship to their formation. I might mention here that on a walk through the hot-houses of the Agricultural Department in Washington, D. C., I had the good fortune to find exactly similar knees, although as yet in a very early stage of development, on the roots of the Durio Zerebenthinus, the well-known Malayan fruit-tree and belonging to a very different family of plants. Some of these which I obtained by the courtesy of Mr. W. Saunders, the chief of the hot-house establishment, show exactly the same development as those of Taxodium, and though very scarce, a fungus with similar spores was found in it. I have written to Dr. Treub, the director of the well known botanical gardens in Buitenzorg, Java, for information in regard to the occurrence of these knees upon the tree in its natural habitat, and his answer may reach me before long.

The material on which this study is based, was collected in the summer of 1892 on the beach of the James river, in Virginia, a short distance east of Newport News. There was but a small grove of Taxodium, covering only a few acres near the beach and a little fresh water was running through it. The water of the river at this point is salt enough to supply oysters to the surrounding inhabitants in great abundance, and those trees scattered along the shore, out of the reach of any fresh water at all, showed the knees equally well. None of the knees here reached a very great size, the largest being perhaps 15 inches above the surface of the soil.

If now one cuts one of these cones close above the soil and takes it home for investigation (and when a knee is sent to you from a distance it usually is cut exactly in this way), one is surprised to find that the cross section of this organ, in other words the section perpendicular to the longitudinal axis of the piece, does not at all answer to the conception one has of a cross section of a Coniferous wood, but in fact shows all the peculiarities of one which is taken

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tangentially from a Conifer. If now a median longitudinal section is cut through the organ, the *general* aspect may resemble what we would expect from a longitudinal section through a Conifer, or it may show us a typical cross section of Taxodium, this only depending on the direction in which the cut has been made. We find, therefore, that two median longitudinal sections crossing each other at right angles are very different, one being a cross, the other a longitudinal section of a Coniferous wood. A radial section between these two shows us the picture of an oblique section. This somewhat startling fact becomes clear to us as soon as we study the development of these peculiar outgrowths. If we dig a cone entirely out of the ground we see that at two opposite sides of it a root extends from it, the proximal one to the tree being thin and about parallel to the surface of the soil, the other the distal one, turning abruptly towards the centre of the earth and being very much thicker.

On hunting for younger stages we soon discover, as we shall see, that these two roots, as they appear to be, are really only part of one and the same. The history of the young root is as follows: It seems that the Cypress forms besides its normal roots, a system of roots starting from the base of the tree and running close to the surface of the soil. Such a young root may run in this way remaining nearly straight for a considerable distance, then it apparently turns suddenly upwards, only to turn down just as abruptly (Pl. XVII, Figs. 1, 2, 3, 4, 5, 6, 7, 9, 10, 11). In this way a bow-like structure results. It is from the most elevated point of this bow, which is either very close to the surface or even a little above it, that the young cone starts. In its first stages this is nothing but an unusual increase at one side.[1]

If now we make at the point where this knee is going to be formed a cross section, we see that here an unusually luxurious, but otherwise absolutely normal formation of wood takes place (Figs. 1, 2, 3, Pl. XVIII). This cross section here, of course, corresponds later to one of the longitudinal sections of the big cone, and now we see also why the two longitudinal sections of the cone are different, as

[1] The history of the development of the young knees to this point has already been described by Wilson in the Proceedings of the Philadelphia Academy of Sciences, 1889, p. 67–69. As at the time of my investigations, I was wholly unacquainted with this article, my own results form a good confirmation of Prof. Wilson's researches.

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the other one, of course, corresponds to the longitudinal section of the root.

It thus appears that the knee is morphologically a limited local hypertrophy at one side of the root, and now we see why the cross section of the cone is in fact a tangential section. This local hypertrophy takes place over a certain distance, but as the growth at one point is very strong and gradually decreases towards the sides, it is clear that cap-like layers of wood ⌒ are gradually superimposed on the one side of the root, a tangential section here, of course, is not purely tangential. How much it will deviate from this depends materially on the steepness of the curve. Simultaneously, of course, the bark at the sides of this outgrowth has been lifted and with it the cambium layer beneath it, so that the young cone is covered by a cambium layer over its whole surface. This cambium near the base of the cone and all over the sides of it, clearly contributes by its growth, to an increase in the thickness of the cone.

Now it is plain that by the growth of this cone at one side of the root, the layers on the opposite side will be folded. It is, in fact, the same thing as if we were to take a rubber tube and thicken one side of it very much; the result being a flaw on the opposite side. This we find here distinctly in the woody layers, and as by this bending the bark becomes compressed, we see this somewhat furrowed just in the angle of the knees. Sorauer has found that when twigs of trees are bent, an abnormal amount of wood is formed towards the inside of the curve, and this we find in the knees also, but on account of the much greater growth on the opposite side, the cone, this is only visible on microscopic examination.

As we have seen already the region of maximal growth is very limited and is found at the top of the young knee. This point of maximum growth, however, does not follow a straight line. In other words, not every succeeding point of maximum growth is perpendicularly above the preceding one, but it moves now a little to the left, then to the right, now towards you, then from you, when you place the knee-root before you on the table so that the longitudinal axis of the root crosses your longitudinal axis at right angles. The result of this is, of course, that a cross section or a longitudinal section does not always appear to be the same throughout. Thus

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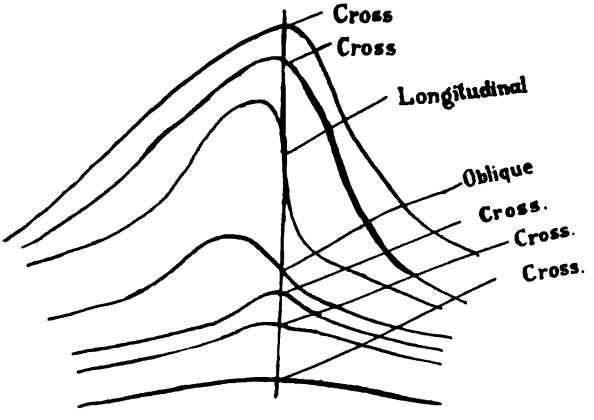
we may get in one cross section a combination of cross-cut, obliquely-cut and longitudinally-cut elements. The longitudinal section may give us for the same reason, a longitudinal, an oblique and a tangential view. In addition to this we find yet another compli-



cation: after a certain time, namely, the cells next to the periphery become entirely turned around. This can be explained, I take it, in this way. By the strong growth at the most elevated part of the cone, the bark and with it the cambium would be pulled over a slanting surface formed by the cap-like layers of wood, unless by an unusual dilatation of the bark this was prevented. Such a dilatation of the bark now takes place near the point of maximum growth where both medullary rays and interstitial tissue have divided their cells repeatedly and continue to do so (Pl. XVIII, Fig. 5). This dilatation is very much less marked farther from the growing point. A considerable friction results from the radial pressure of the bark. If now the growing point was always in a line perpendicular to the longitudinal axis of the wood-fibres, this transplacement would only be parallel to this axis, and at the utmost a rupture would take place. If the force was not sufficient for this a somewhat broader cell might perhaps result. If, however, as is the case here, the point of maximum growth moves a little further backwards or forwards the direction of the pulling force is no longer perpendicular to this longitudinal axis of the fibre, but forms an angle with it and the result is that the new fibre comes to form an angle

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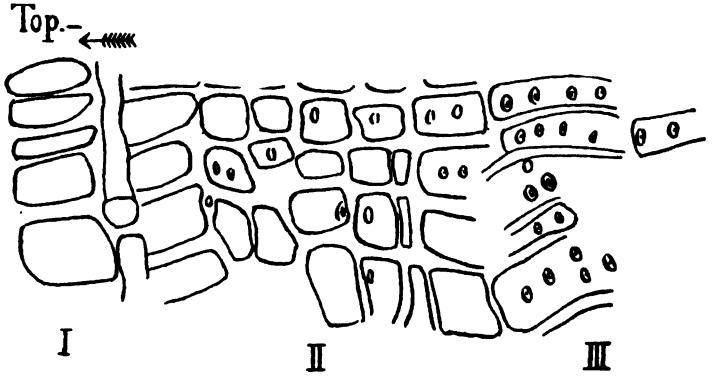
with the original one and finally becomes entirely turned around, so that we see the radial pitted walls in front view on cross section. The parts below and above this turning point may yet continue their normal growth as seen in the accompanying figure, and in Fig. 3, Pl. XVIII.



If we cut now from such a piece of the cone, a cross section following the radius, we get a section as in Fig. 4, Plate XVIII. Here the parts I and I, both representing cross sections of wood (only partly drawn) and bark were once close together, only separated by the cambium. All that which is now between it is formed later on. To parts II and II the obliquely-cut fibres of wood and bark, respectively, were close together also, and the longitudinally-cut parts III are the last formed ones. So we see that up to the time of the formation of II everything was normal, during the time that II was formed only a begin of turning had taken place, and it was not before III was formed that the turning around of the fibre was completed.

We see then that the longitudinal axis of the fibre, instead of crossing the longitudinal axis of the cone as formerly, is now parallel with it. This now may proceed more or less regularly (Plate XVIII, Fig. 3) from the base towards the top, always, however, leaving the real growing point free, so that finally the cone is surrounded with a mantle of longitudinal elements parallel to the longitudinal axis of the organ (Plate XVII, Fig. 18), above, which only the growing point peeps out. (Near this growing point we often find chlorophyll

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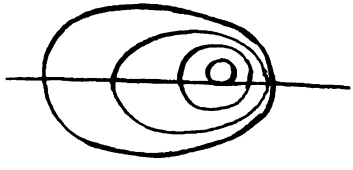
in the bark, not an unusual appearance in roots exposed to light). So that now we find that the longitudinal section near the surface of the knee is a genuine longitudinal section in whatever radial direction we cut.

The cross section of the peripheric layers is then also a genuine cross section; and the tangential one, a real tangential section, so we get on a cross section of the older knee at the periphery a real cross section, and farther towards the centre an oblique or even a tangential one according as it happens that the layers are more or less bent and the line which unites the growing points is more or less crooked. The longitudinal section offers the picture which we have seen already.

Afterwards the cambium proceeds to form, at the periphery, longitudinal elements, starting from the base and proceeding towards the top, and so we get, surrounding the cone, a thick mantle (Plate XVII, Fig. 18) of longitudinal elements which in old knees may form by far the greatest part of the knee.

On longitudinal sections we sometimes see near the center of the old root a tangentially cut portion. This may be explained by the fact that the growth of the knee did not take place symmetrically in regard to the longitudinal axis of the original root, but was more or less one-sided, so that the longitudinal section of the knee cut the original root tangentially.



Occasionally, not only the fibres are twisted, but also the whole organ (Plate XVII, Fig. 12), in this case the twist may reach as in the pictured knee 45°, and the result is that the two longitudinal sections are now the same. Both give, of course, an oblique section.

Besides these knees, which we might call the typical knees, I found, as Wilson did,[1] other knees, namely those where a thickning had taken place over a much greater distance, so that no real cone

[1] Wilson, l. c.

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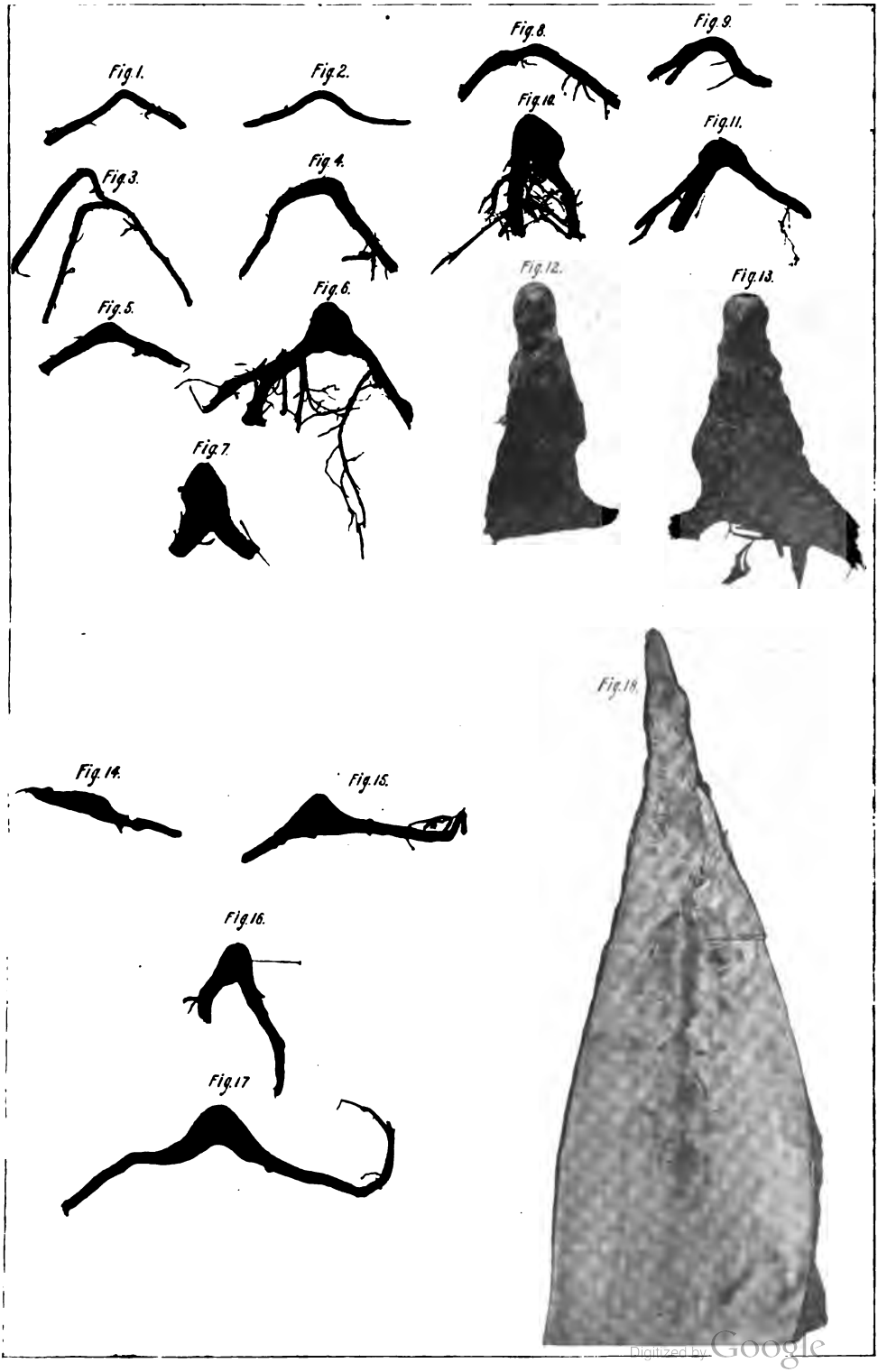

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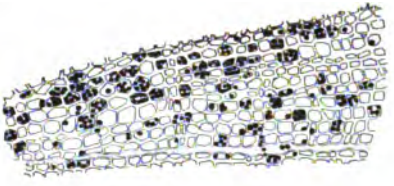
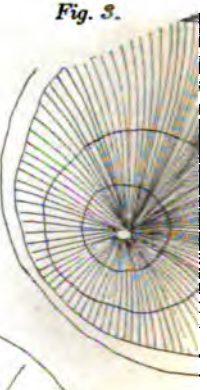


Fig. 7.

Fig. 3.



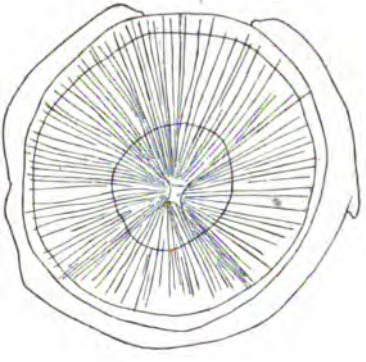


Fig. 1.

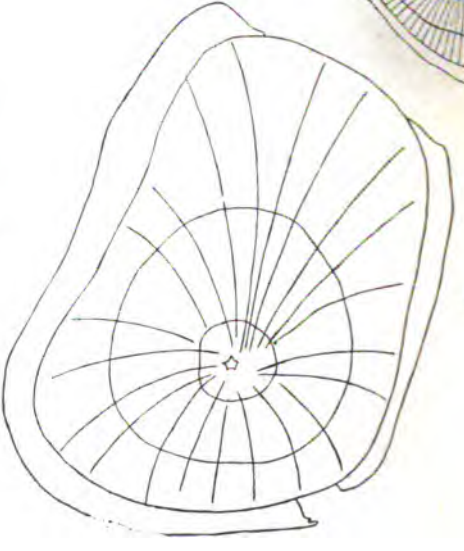
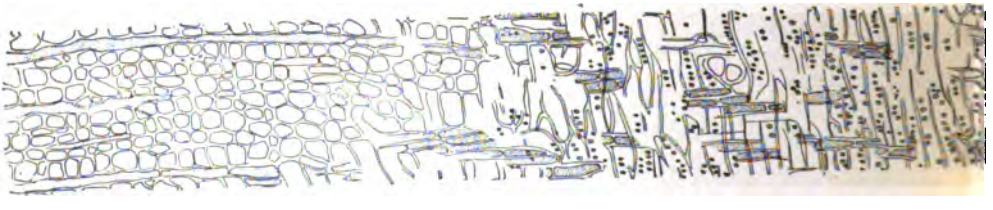


Fig. 4.

Fig. 2.



I. *II.*

J. P. Lotsy, Autograph.

TAXODIUM

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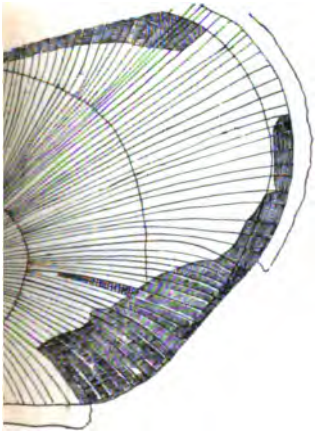
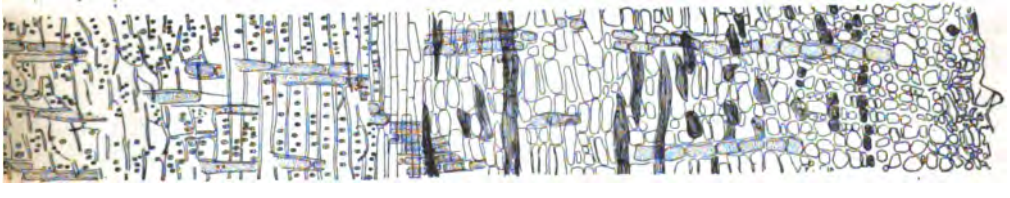


Fig. 5.

Fig. 8.

Fig. 6.



III. *C.* *III.* *II.* *I.*

DISTICHUM.

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formation had taken place, but the root was simply bended and thickened over a large area; secondly, a root may first start in this way, but afterwards form a cone, which is then elevated above the thickened portion, and thirdly, two roots which have started the formation of cones may grow together and so form a cone of two stories.

BALTIMORE, J. H. U., *May 15th*, 1893.

EXPLANATION OF PLATES.

PLATE XVII.

Figures 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13. Different stages in the development of a knee of the Taxodium distichum.

Figures 14, 15, 16, 17. Same of Durio.

Figure 18. Cross-section through an old knee of Taxodium showing layers of wood parallel to the longitudinal axis of the organ superimposed by the cambium after the fibre has been turned around.

PLATE XVIII.

Figure 1. Cross-section through young root showing the very first swelling. In this and the two following figures the absence of the bark at some points is due to it having been cut away by the knife.

Figure 2. Cross-section through a little older root.

Figure 3. Cross-section showing the next stage, the fibre is beginning to turn around, it is turned around in the shaded parts.

Figure 4. Cross-section of wood and bark following a radius like a from a stage like Figure 3.

Figure 5. Cross-section of bark in Figure 3 at point C.

Figure 6. Longitudinal section of root in stage of Figure 1, showing spores of fungus as black dots inside of the cell.

Figure 7. Corresponding cross-section to Figure 6.

Figure 8. Cross-section near top of a young cone, showing hyphe growing through pit in cellwall and forming spore at top.

All figures are drawn with the camera lucida. Figure 1, 2 and 3, are somewhat schematic.

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ON THE ORIGIN AND DEVELOPMENT OF THE STICHIDIA AND TETRASPORANGIA IN DASYA ELEGANS.

By B. W. BARTON. April, 1893.

In the literature bearing upon the Florideæ which we have been able to consult, there were found no descriptions and no figures giving in detail the development of the Stichidia. Agardh in his well known paper "Floridernes Morphologi," published in the proceedings of the royal academy of Sweden at Stockholm (Kongliga Svenska Vetenskaps—Akadamiens Handlingar—Ny följd. Femtoride bandet, 1877) devotes a small space to the subject and gives in the accompanying atlas a fragment of a figure presenting a longitudinal section of a stichidium of Dasya elegans which, however, does not accord with what is here to be described.

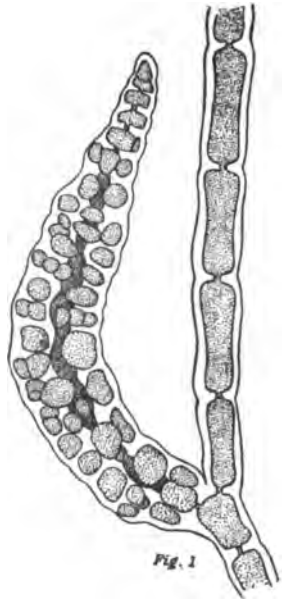

Fig. 1

The stichidia appear as spindle-shaped, many-celled objects scattered along the axes of the mother plant without regard to any apparent order of relation to the vegetative shoots. Here and there among the numerous false dichotomies, one cell is diverted to the formation of a stichidium whilst the other carries on the further growth of the frond. One learns to distinguish the two sorts of branches at an early period of development by almost undefinable differences, the cells of the stichidial branches being rather more compact and more closely jointed than those which are purely vegetative or conductive.

The stages in the development of these specialized branches which bear the tetrasporangia may be followed out in any such branch

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still in process of active growth. The order of development is acropetal, and whilst near the base the sporangia may have reached maturity or even cast off the tetraspores, others are to be seen in the various formative stages along the course of the branch from the middle region on towards the apex. Fig. 1.

The apical cell is conical and is ever growing forward whilst a new axial cell is continually being lopped off by a transverse wall near its base. The column of cells so formed move apart but retain connection with one another by means of a thin thread of protoplasm making it appear that they had arisen by simple circular constriction, but not severed from the mother cell. Fig. 1.

At a distance of a few cells behind the apex, these axial cells undergo concentric or tangential division, in such a manner as that there arise a central cell and four or five lateral or radial cells which are at first approximately the same shape. Fig. 2. As they increase in size and move apart, as if by the insertion of the gelatinous layer, the radial cells are seen to be quite disconnected the one from the other, but each is still holding to the central cell by the characteristic protoplasmic thread. The central cells became more and more drawn out at either end by the continous growth in length

Fig. 2.

Fig. 3.

Fig. 4.

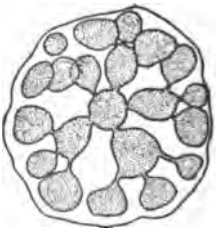

Fig. 5.

of the stichidium and by the pressure of neighboring cells and come at last to take the form of well-marked spindles from whose thickest parts radiate the lateral cells. Figs. 1, 3 and 6. These latter having in the meantime attained considerable size are divided by a horizontal radial wall, that is by a wall at right angles to the axis of the stichidium, into an upper and lower cell. Fig. 4. The upper one is the tetrasporangium or tetraspore mother cell. The lower

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one grows somewhat in length and divides at first tangentially, then radially so as to give rise to three surface or peripheral cells. These remain attached to the radial cell but have no threads of connection with each other. The tetrasporangium likewise retains its hold upon the same radial cell. Fig. 6.

The protoplasmic thread connecting the cells of the many systems which make up the stichidium, furnishes a means of tracing the line of descent of any given cell in any system. Thus the members of each group of three peripheral cells are only sisters descended from a common mother radial cell and are the granddaughters of the axial spindle cell, these latter being the immediate offspring of an axial cell.

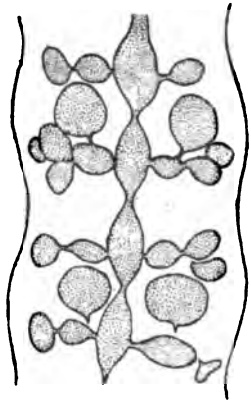

Fig. 6.

The persistent constancy of the number three, the peripheral cells, daughters of the lower division of the primary radial cell, and the equal constancy in the appearance of the brood of four in the upper or sporangial cell does not quite justify the assumption of homologies in these two groups, although the zoölogists do not hesitate to assume homologies in analogous instances, as when a germ cell with its three polar bodies are accepted as the homologues of four spermatozoids the offspring of a single sperm cell.

Near the middle and larger region of the stichidium the peripheral cells often further subdivide and so increase in number round about the sporangium cell as nearly to enclose the latter but in turn are crowded and forced into sundry shapes by its rapid growth to a large size. The sporangium may be seen supported upon its pedicel always on the upper or distal side of its sister cell, Fig. 6, and not in pairs as figured by Agardh, the one above and the other below, both attached to the small radial cell. As a rule but two sporangia are seen, and frequently only one sporangium is borne at any given node, although the limitations are not very strict in this respect. It is invariable, however, that but one sporangium accompanies any given radial cell.

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When the spores escape, the circular opening amidst the peripheral cells looks into a spherical cavity now occupied only by the ragged trabeculæ of the tetrasporangium. Near the base of the stichidium division ceases with the formation of the primary radial cells which thus thicken and strengthen the pedicel of the organ. Fig. 1.

Occasional instances of prolification are met with, in which the apical cell appears to grow more rapidly, lopping off behind it the row of axial cells which instead of subdividing undergo no further change than that of increasing in length and assuming the general characters of the vegetative frond.

The process of parafine imbedding caused some shrinkage of the tissue and unnatural approachment of the cells as indicated in the drawings of the sections when compared to that of the stichidium which is taken from a specimen preserved in dilute glycerine and alcohol.

Thanks are due to Dr. Lotsy for furnishing material used in this study and for aid in reviewing the literature of the subject.

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FROM THE

BIOLOGICAL LABORATORY

CONTENTS OF VOLUMES I-V

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